Environmental Studies No. 24

Effect of Crude Oil on Polar Bears

Northern Affairs Program

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and P.D. Watts

©Published under authority of the Hon. John C. Munro,
P.C., M.P., Minister of Indian and Northern Affairs,
Ottawa, 1981.
QS-8283-020-EE-A1
Canada Catalogue No. R71-19/24-1981E
ISBN 0-662-11835-9

This report was prepared under contract for the Northern Environmental Protection Branch, Indian and Northern Affairs Canada, as part of the Eastern Arctic Marine Environmental Studies (EAMES) Program. The views, conclusions and recommendations expressed herein are those of the authors and not necessarily those of the Department.

A Canadian contribution to the Unesco Program on Man and the Biosphere (MAB).

Cette publication peut aussi être obtenue en français.

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1. SUMMARY

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1.1 Rationale

It is widely perceived by the general public that oil spills and oil well blowouts will occur in Arctic marine areas as a result of offshore petroleum development and that these may have detrimental effects on wildlife populations. The actual extent or severity of such accidents particularly with respect to Arctic environments is not, however, well understood.

The polar bear (Ursus maritimus) is, at present, not considered an endangered species. Its position on top of an ecological food chain, however, determines that the population remains at a low level relative to seals, its main food source. Assuming that hunting quotas and methods will continue to be regulated according to results of contemporary population research, it is the increased human occupation and pollution of polar bear living areas that present the greatest threat to survival of this species.

An investigative study was designed to simulate an arctic oil spill and determine its effect on polar bears experimentally exposed to the crude oil, a Midale crude. The impact of coating was to be assessed particularly on thermoregulation in the polar bear, with effects related to the characteristics of the oil, its changes with exposure, and its uptake from external coating as measured by plasma residue levels. An ongoing health program would monitor any shifts occurring in stress indices. As a consequence of the unexpectedly severe toxicity effects of oil exposure on the bears, it became necessary to extend the study program to include further assessments of hydrocarbon residues in body tissues and fluids, as well as a more extensive clinical assessment, and a detailed post-mortem examination of the two polar bears that died. Recovery of one bear was effected, and monitored closely to assess treatment possibilities for future accidental exposures.

1.2 Thermoregulation Effects

The metabolic and temperature responses of three polar bears were measured before and after exposure to crude oil. Three experimental arrangements were used:

(a) Two fans created wind speeds of 5 m/s directed at a small holding cage,
(b) an open circuit respiration chamber was used for indirect measurements of resting metabolic rate (energy turnover), and

(c) another open respiration chamber, mounted on a treadmill, was used to determine the metabolism at a walking speed of 5.4 km/hr.

Body and ambient temperatures were recorded throughout all aspects of the study. Due to mechanical breakdown of the treadmill, walking metabolism after oil exposure was tested in only one bear.

Oxygen consumption was determined by use of an electronic oxygen concentration analyzer and a standard bell or dome gas meter. Body temperatures (deep body and skin) were determined by means of surgically implanted temperature radio-transmitters. Oil exposure was accomplished by enticing the experimental polar bears into a small seawater pool covered with a 1 cm thick layer of Midale crude oil.

Complementary studies of the thermal properties of polar bear fur were performed on normal and oil-coated fur samples.

A computer model of polar bear heat balance was developed, partly through the present project. The model was tested for applicability to polar bears in the wild by using the results from the present work. The model was also used in the interpretation of the results of oil exposure.

Prior to oiling, all three polar bears exhibited normal thermoregulatory responses: During wind tests, skin temperatures dropped, reflecting the anticipated reduction in peripheral blood circulation. In the respiration chamber, the metabolic values were in reasonable agreement with Kleibers formula for the basal metabolism of mammals, $M = 3.4 W^{0.75}$, where W = body weight (kg). Deep body temperatures of the resting polar bears averaged near 36.5°C which is in good agreement with previously reported results.

The measured metabolism of polar bears on the treadmill was higher than predicted by general formulas of activity metabolism as derived from other species. The observed metabolic values in combination with results from previous polar bear studies were analyzed; the resultant straight line relationship allowed generation of a new equation of polar bear activity metabolism, for standard conditions.

The increase in deep body temperature following the 30 minute treadmill tests was in the range of 1.4 to 2.2°C; increments predicted by the computer simulations when the new equation for activity metabolism had been included in the model. Also in agreement with the model predictions, was a positive correlation between the deep body and skin temperatures of the exercising polar bears.

The polar bears, when confronted with crude oil, showed no obvious aversion to it, and actively ingested oil by licking it off their fur following the actual exposure. Extrapolations from the available behavioural observations, to prediction of the behaviour of bears in response to oil under natural conditions, however, would be premature.

After oil exposure, considerable increases in both metabolism and skin temperatures were recorded. For resting polar bears the metabolic rate was nearly doubled. This increment, when considered together with the more than doubled heat conductance values of the oil coated fur samples, was interpreted as a normal and significant thermoregulatory response. The skin temperature increments were abnormal, however, and were interpreted as indicative of a skin reaction to oil.

For the post-oil exercising polar bear, the metabolism was higher than expected, both for thermal balance and work load. At the same time, the deep body temperature increased in a fashion reminiscent of fever in man.

The skin temperatures of the post-oil polar bear were abnormally high prior to exercise and showed minor increments during the work load test, indicating that an established skin reaction was over-riding normal thermoregulatory responses.

It is clearly established that oil fouling will cause severe cold stress of polar bears. Cleaning the fur of the experimental animals was visually successful, but also raised concern regarding the timing and the possible disadvantages inherent in drugging weakened polar bears.

The observed thermoregulatory responses can be assumed to alter polar bear behaviour. The potential effect of oil exposure on the feeding habits of polar bears are briefly discussed, both with respect to possible changes in hunting behaviour and to direct effects on the primary food source.

The computer model presented here may be used for quantitative assessments of the severity of eventual exposure of polar bears to crude oil. Oil pollution may become a serious threat to the survival of this species.

1.3 Oil Characteristics, Accumulation, and Clearance

This part of the study has demonstrated that Midale crude oil resembles arctic oils in a number of respects, being of medium viscosity and showing a wide range of hydrocarbon fractions. This included benzenes, naphthalenes and paraffins, which were standardized in analysis.

A test of weathering effects by air and water showed that relative shifts occurred from the lightest molecular weight fractions to medium weight hydrocarbons. No major changes or losses were found relevant to the experimental period.

The Midale crude was shown to have characteristic UV-fluorescence and UV-absorption spectra. These were used for quantitative and qualitative assays on all samples, as were gas chromatographic assessments on selected samples.

Oil was found to be present at high concentrations in vomitus and feces after the exposure period, and at lower concentrations in feces and stomach contents throughout the post-oiling period. Continued exposure is suggested, originating from grooming activities of the fur which retained large molecular weight hydrocarbons in basal layers.

Plasma showed an accumulation of oil, maximum after exposure and slowly declining in concentration thereafter. Tissue types showed a differential oil residue accumulation.

Urine and bile were identified as major routes of release of the oil load in the polar bears. Urine and bile showed high levels of oil soon after dosing, and urine continued to carry oil residues for the entire post-dosing experimental period.

1.4 Hematological and Biochemical Toxicology

Exposure of polar bears to crude oil had as major sequelae, erythropoietic dysfunction and renal abnormalities. Although the initial symptoms of these effects appeared early, the maximum response was latent, requiring 5 to 6 weeks to develop. These two conditions were marked by a microcytic hypochromic anemia, and by elevations in serum bilirubin, urea, creatinine, uric acid, and inorganic phosphate, while a hyponatremia resulted from loss of Na^+.

White blood cell parameters were little affected, as was serum glucose. Cholesterol elevations were noted towards the end of the oil exposure period. Hepatotoxicity was minimal, showing rises in diagnostic enzymes only soon after the oil exposure, which then declined.

Some hormone changes were observed. An early stress response in cortisol was followed by a decline to below normal levels in plasma. There had not, however, been a loss in response capacity since all bears showed major elevations at the critical terminal period. Of the two thyroid hormones examined, T_3 showed a different oil-related response from T_4, with elevations evident as compared to control values. Plasma T_4 levels declined at the end of the experimental period in all bears.

Recovery was followed in the surviving animal, Bear 2. Recovery was complete five months after the initial exposure, 3-1/2 months after the start of therapy.

1.5 Pathology

The crude oil related effects were polysystemic and the renal changes were the most serious under the laboratory conditions. The acute renal insufficiency was identifiable in Bear 4 only after death, terminally in Bear 3, and early enough in Bear 2 for therapeutic measures to be effective.

The experimentally oiled polar bears suffered acute renal insufficiency that most likely was of both prerenal and renal origin. Changes in the blood biochemistry indicated severe uremia, dehydration and hemoconcentration. Serum electrolyte abnormalities consisted of loss of sodium and chloride and terminally markedly elevated serum potassium in Bear 4. Absolute and relative elevation of serum inorganic phosphorus coincided with retention of weanitrogen and creatinine in circulation. Moderately severe nephrosis, hemoglobinuric nephrosis, and secondary changes due to uremia dominated the renal histologic picture of the two dead bears. Lesions related to the uremia and severe dehydration were also present in the upper digestive tract, particularly tongue and oesophagus, and the gastrointestinal tract. These lesions consisted of ulcerative glossitis, erosive oesophagitis, gastric hemorrhage and enteritis. Mild to moderate degenerative changes in the brain and the liver of the two autopsied bears were due to dehydration and uremia. Non-regenerative anemia associated with extravascular hemolysis and gastric hemorrhage was seen in all three bears. The anemia was most severe in Bear 2. The lack of bone marrow erythropoietic response was attributed to the myelotoxic effect of benzene present in the weathered crude oil. Acute infectious inflammatory changes were seen in some of the peripheral lymph nodes and pancreas of Bear 4, and abscesses were seen in Bears 3 and 2. The infectious process was iatrogenically induced and the extent of it may have been due to a subnormal leukon response. Bone marrow and the lymphoid tissue were moderately atrophied. The zona fasiculata of the adrenal cortex was hyperplastic and there was a variable degree of degeneration of the zona arcuata.

The lungs were partially atelectatic and the histological findings were suggestive of surfactant deficiency, probably as a result of the effect of crude oil hydrocarbons on the lung tissue. Marked epidermal and hair follicle hyperkeratosis in two bears, and localized alopecia in one, and an acute inflammatory condition of the nasal passages, were probably due to local irritant action of the crude oil.

1.6 Conclusions and Recommendations

A general conclusion which may be drawn from this study is that the polar bear is a potentially greatly impacted species when exposed to oil spills. An initial effect of coating with oil is that thermoregulatory and metabolic stresses develop which may cause serious disability if protracted in the wild. Oil fouling of the fur led to grooming and licking of the oil from the fur, with consequent ingestion of the oil, and absorption into the body from the gut. Residence of oil in the fur may be expected to be long if the animal is not cleaned completely, prolonging exposure by grooming/ingestion activities. Uptake of petroleum hydrocarbons and their distribution to body tissues led to behavioural abnormalities, including anorexia, as well as to tissue damage. A wide range of tissues were found to be affected, much of the effect related to uremia and severe dehydration. Peripheral hemolysis and a lack of bone marrow erythropoietic response resulted in an acute anemia in all oiled bears. The systemic toxicity effects were latent, not becoming pronounced until weeks after the initial exposure. Renal changes were the most serious under the laboratory conditions and can be assessed as the direct cause of death in two of the three oil exposed polar bears.

On the basis of the information available on the crude oil effects in the polar bears as a result of this study, it could be stated with a reasonable degree of confidence that it would be very unlikely that polar bears would again be lost from similar exposure to crude oil under the laboratory conditions.

Insofar as exploration for oil and other oil related activity is a necessary reality in the Arctic, so is the possibility of oil spills. Therefore, information is needed that would form a basis for rational therapeutic and other contingency measures to deal with oiled bears in the wild, possibly many of them at the same time. Without this information we could be faced with a dilemma: whether to treat and possibly prolong the misery, or needlessly destroy many bears that otherwise could be saved with effective therapy if we were more knowledgeable about the oil effects. This leads to recommendations for further studies to be able to assess the total impact of oil spills on this species.

Priority should be given to studies of polar bear behaviour (including physiological indices) in response to a natural, but oil-fouled environment. A large enclosure is envisioned for such studies, facilitating observation in conjunction with monitoring by implanted radiotransmitters. A comprehensive study should assess the polar bears' reaction to oil, to oil on fur, and to oil-contaminated seal offered as food.

Concurrent laboratory studies should be performed, with the objective of resolving the problems revealed by this study concerning potential fever and skin reactions to crude oil. The question as to whether oil may cause fever in polar bears is recognized but is not fully analyzed in the present report. An eventual fever effect of oil has important implications for handling procedures of oil-contaminated animals, however, and should be pursued by further experimentation.

Priority should also be given to study of the thermal properties of fur samples coated with oil and subjected to cold air temperatures.

Although this study has identified that oil spill exposure is detrimental to polar bears, it has not been able to address in any way the possible effects of oil spill dispersants. The use of oil dispersants may be anticipated in a spill situation, particularly if such an accident were to occur in a sensitive area.

Other recommendations may be made for administrative action.

Governmental agencies involved in development of oil resources in the Arctic should spearhead the formation of an independent committee, with the mandate of developing contingency plans to deal with potential oil spills or blowouts. The committee should also review existing information and suggest research priorities designed to anticipate and minimize the environmental effect of oil exploration on polar bears. The committee would include system biologists and physiologists, but should also be fortified with individuals having expertise in toxicity and the logistics of arctic operations.

2. GENERAL INTRODUCTION

F.R Engelhardt, N.A. Øritsland, and R.J. Hurst.

An increasing recent emphasis on exploration and exploitation of arctic petroleum reserves has created a concern at all levels of interest associated with these activities, over their effects on sensitive ecosystems and species. An easily identified area of concern is that of the effects of petroleum spillage on arctic fauna. The potential for petroleum spillage is high, particularly for polar continental shelf reserves, because of the hazardous conditions of production and shipping in arctic waters (Glaeser 1971). These considerations have led to numerous workshops and symposia, as well as comprehensive publications on the subject (see, for instance, Malins 1977).

From the point of persistence of the oil and its toxicity effects, the environment itself presents a problem. Whereas the rate of change, or weathering, of crude oil in a marine environment depends on oxidation, evaporation, dissolution, and biological degradation, the low temperatures of arctic waters tend to decrease the activity of these processes. In particular, the rate of biological oxidation may be expected to be low. Further, the low temperatures retard volatilization of the oil, including that of the light fractions common to arctic petroleum. These fractions have been shown to have marked toxic effects in many organisms and their effective toxicity will be enhanced (Barnett and Kontogiannis 1975, Mackay and Shui 1976, Morrow 1973, Ottway 1971). In addition, ice conditions will modify the pattern of spreading and disappearance of an oil spilled in arctic waters (Glaeser 1971, Snow and Scott 1975). One effect of a broken ice cover is that the oil may concentrate in leads and breathing holes. Wind may act further by "herding" of the oil between moving ice-floes, or against an ice edge (Ayers *et al*. 1974). The potential of this action in forming a hazard to arctic marine mammals has been suggested (Engelhardt 1978), but mainly with respect to potential problems in seal species. Little consideration has been given to polar bears (*Ursus maritimus*), the species of concern in this study. The specializations of cold climate animals such as the polar bear, and their seasonal concentrations, make them vulnerable individually and as a population to any sizeable oil spill.

The polar bear may be considered to be a marine mammal in the general sense of the term, and more specifically, as far as its habits, habitat and potential for petroleum impact are concerned, is analogous in many ways to seal species. In that sense, the polar bear may show effects of oil toxicity similar to those reported in seals. In seals, exposure has been cited to cause eye irritation (Nelson-Smith 1970, Smith and Geraci 1975) and other damage (California Department of Fish and Game 1969, Lillie 1954, Spooner 1967). The Santa Barbara Channel oil spill has been suggested as the cause of mortalities in island colonies of California sea lions (*Zalophus californianus*) and northern elephant seals (*Mirounga angustirostris*). However, Brownell and Le Boeuf (1971) interpreted these as natural mortalities. Le Boeuf (1971) also reported that elephant seal pups recovered from oil coating. Reports of exposure of arctic seals to spilled oil show that no related mortalities occurred following contact (Hess and Trobaugh 1970, Morris 1970, Muller-Willie 1974). The most comprehensive study on the effects of oil on marine mammals has been one carried out in the Beaufort Sea in 1974 (Beaufort Sea Project) and 1976, reported variously by the joint work of Smith, Geraci and Engelhardt, on ringed seals, *Phoca hispida*. Smith and Geraci (1975) and Geraci and Smith (1976) reported behavioural, physiological, and pathological effects of crude oil in the ringed seal, *Phoca hispida*, as part of an impact study of oil spills in the Beaufort Sea. In ringed seals, exposure by immersion to Norman Wells crude oil resulted in transient problems of eye irritation, minor kidney damage, and possible liver lesions when carried out in the field, and in the death of the exposed animals when immersed in a laboratory study. No effects on body-core temperature were noted. Ingestion of the oil caused no significant lesion or behavioural changes. Ringed seals showed rapid absorption and an effective metabolism and clearance of petroleum hydrocarbons from the experimental oil, whether exposed externally or orally through oil contaminated fish (Engelhardt et al. 1977, Engelhardt 1978).

There are several reasons why polar bears may be considered a sensitive species with regard to petroleum pollution. The suggestion that the magnitude of an oil impact on polar bears may be major has been put forth (Birchard et al. 1978). Polar bears spend a great amount of time travelling across sea ice and traverse open water leads in search of food, primarily seals (Øritsland 1969). They then become particularly susceptible to under-ice spills and the effects of "herding" of oil by wind and ice action (Ayers et al. 1974), as well as to oil spreading on top of the ice. The possibility of ingestion of oil fractions is real, in that ringed seals have been shown to accumulate petroleum hydrocarbons (Engelhardt et al. 1977, Engelhardt 1978). Polar bears feed predominantly on ringed seals throughout their range (Stirling and McEwan 1975). Muller-Willie (1974) reported that ringed seals in Hudson Bay have been observed to be completely covered with oil. As the top member of the animal food chain, the polar bear may then actually receive high inputs of bioaccumulated petroleum hydrocarbons following an arctic oil spill.

Although seals show no thermoregulation effect by coating of the external hair coat (Smith and Geraci 1975, Geraci and Smith 1976, Øritsland and Smith 1975), the situation in the polar bear may be different in that the animal does rely on the thermoinsulative properties of the fur. A preliminary study by Øritsland (1976) demonstrated some shift in insulative properties of the oiled fur.

No less a consideration than the strictly biological ones above is the fact that the polar bear is an arctic species which is of great import to northern native peoples. It has social and religious connections, at least historically, and still serves as an economic consideration to native settlements to the limited extent to which it is hunted.

The CITES classification of the polar bear as a threatened species was a major contributing factor to the necessity for assessment of the effects of petroleum pollution in this species.

An experiment was designed to simulate an arctic oil spill to determine the effect of crude oil coating in particular on thermoregulation in the polar bear. The impact of coating on thermoregulation was planned to be related comprehensively to the characteristics of the crude oil in question, its changes with exposure, and its uptake into the bear as measured by plasma oil residue levels. An ongoing health program would monitor any shifts occurring in stress-related parameters. As a consequence of the unexpectedly severe toxicity effects of oil exposure to the bears, it became necessary to extend the study program to include further assessments of hydrocarbon residues, a more extensive clinical assessment, and a detailed post-mortem examination of the two polar bears that died.

3. EXPERIMENTAL OUTLINE

N.A. Øritsland, R.J. Hurst, P.D. Watts, and F.R. Engelhardt

3.1 Simulated Spill Studies

3.1.1 Study Animals.

All animals used in this study were sub-adult polar bears captured in the vicinity of Churchill, Manitoba, Canada. Four individuals, deemed "problem" or "nuisance" bears, were obtained by personnel of the Manitoba Department of Renewable Resources by the use of culvert traps.

The three animals subsequently exposed to oil and experimentation were 2 males (Bears 3 and 4) and 1 female (Bear 2). They were of the same general age (2 - 3 years) and size (145 - 165 kg), and were captured and moved to the Churchill Polar Bear Laboratory between October 09 and November 03, 1979.

The bears were aged by Dennis Andriashek, C.W.S., Edmonton, Alberta, using the tooth cementum annuli technique described by Stoneberg and Jonkel (1968). A weighing scale was used to obtain weight at time of capture. Weight measurements were repeated throughout the holding period at any time the animals were immobilized.

A fourth bear (Bear 1) was captured on August 05, 1979 and held at the Churchill facility until March 25, 1980, at which time it was flown to the Assiniboine Zoo, Winnipeg, Manitoba. Its use in the oil exposure study was rejected in March, 1980, due to the animal's unusual and potentially dangerous tolerance to immobilizing drugs.

At capture and movement into the lab of all bears, the immobilizing drugs Sernylan (phencyclidine hydrochloride, Bio-Ceutic Labs. Inc., St. Joseph, Missouri) and Sparine (promazine hydrochloride, J. Wyeth and Brother Ltd., Windsor, Ontario) were administered.

While in captivity the bears were immobilized for handling and surgery using Ketaset (ketamine hydrochloride, Rogar/STB, BTI Prod., London, Ontario) and Rompun (xylazine hydrochloride, Baychem. Corp., Kansas, Missouri). A meter long jab stick was used to inject the drug solution rather than subjecting the animals to the trauma of a dart gun. A solution containing 2.5mg of each drug/lb of body weight (5.5 mg/kg) was administered in each initial dosage. Additional hand injections were given if immobilization had to be prolonged. For an animal in a weakened condition or for a move requiring little handling, the initial dosage was occasionally reduced to 2.0 or 1.5 mg/lb of body weight (4.4 or 3.3 mg/kg). Appendix 5 details the immobilization of the bears.

All animals were fed a diet of beef scraps obtained from an abattoir, occasionally supplemented by meat and blubber of ringed seals. Water was supplied ad libitum until November when laboratory temperatures precluded use of water in favour of snow, also ad libitum. On completion of the majority of experimentation (Mar. 06) water was again offered and the amount recorded.

3.1.2 Experimental Facilities

Holding cages

All in vivo experimentation was carried out in the Polar Bear Project Laboratory in Churchill, Manitoba. Study animals were kept separated at all times. A minimal number of druggings and easy movement of bears was afforded by the heavy steel construction, which occurred in the year prior to February, 1980.

Before 1979, facilities included two holding cages (3.7 x 2.4 x 1.5 m) adjoined by a walkway to the treadmill, and one holding cage (3.7 x 1.8 x 1.4 m) located in a separate enclosed room. Construction in 1979 of a separating wall in the largest cage to form two separate cages (3.7 x 1.8 x 1.4 m) allowed for all bears to be adequately held until the new cage complex was complete. New cages were designed from panel sections measuring 1.2 x 1.2 m and 1.2 x 1.5 m. Each section was made of square steel tubes (5 x 5 x 120 cm) welded at each end to angle-iron at 15 cm intervals. Sections were joined by both bolts and welds.

The new cages included two holding cages (3.4 x 2.4 x 1.2 m) and one multi-purpose cage (recovery/wind exposure/behaviour) measuring 2.4 x 1.2 x 1.2 m. The floor was raised 0.5 m from the cement floor to prevent accumulation of faeces, facilitate cleaning, and allow connection with the two existing cages. The resulting complex of five cages allowed movement, via a series of walkways and sliding doors, between the individual cages. It also provided access to both the treadmill and the pool. See Figure 3-1.

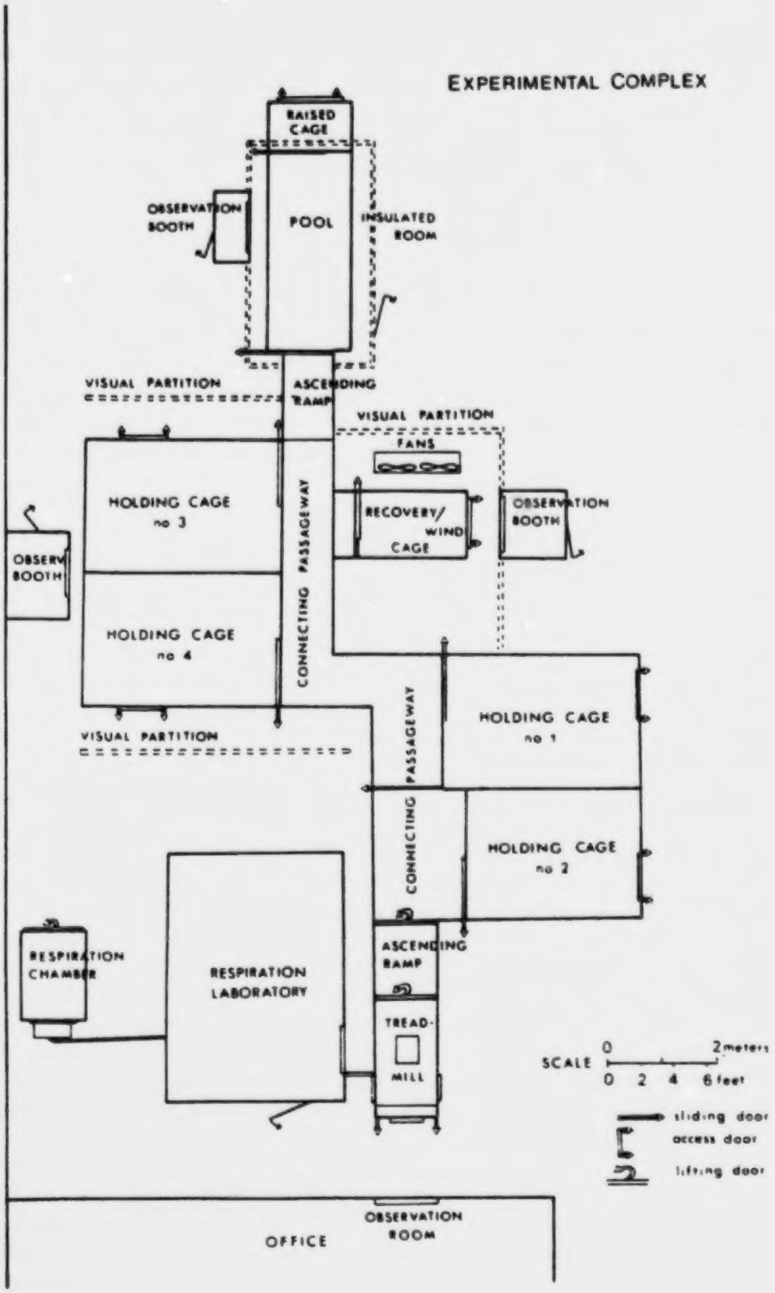

EXPERIMENTAL COMPLEX
RAISED CAGE
OBSERVATION BOOTH
POOL
INSULATED ROOM
VISUAL PARTITION
ASCENDING RAMP
VISUAL PARTITION
FANS
HOLDING CAGE no 3
CONNECTING PASSAGEWAY
RECOVERY/ WIND CAGE
OBSERVATION BOOTH
OBSERV BOOTH
HOLDING CAGE no 4
HOLDING CAGE no 1
VISUAL PARTITION
CONNECTING PASSAGEWAY
HOLDING CAGE no 2
ASCENDING RAMP
RESPIRATION CHAMBER
RESPIRATION LABORATORY
TREAD-MILL
SCALE 0 2 meters
0 2 4 6 feet
sliding door
access door
lifting door
OFFICE
OBSERVATION ROOM

Figure 3-1. Experimental complex used at the Polar Bear Project Laboratory, Churchill, Manitoba. All structures north of holding cage 1 (i.e. holding cages 3, 4, pool etc.) were constructed in 1979-80 in preparation for this study.

Pool

A pool was designed using 1.2 x 1.5 m 10 gauge steel plate. Plates were welded at seam joints and strengthened by a framework of bars and angle-iron. At the 1.5 m level, panels identical to those used for holding cages were welded to form a cage above the pool level.

The resulting structure measured 3.7 x 1.5 x 3.0 m with a pool of 3.7 x 1.5 x 1.5 m and a water containment volume of 6,800 l (1,800 gal.).

A small access cage (1.5 x 1.2 x 1.2 m) elevated to the 1.5 m level was supported at one end of the pool. The opposite pool edge was connected by a descending ramp to the walkway adjoining the holding cages (Figure 3-1).

A room was constructed around the pool primarily to insulate the water from cold laboratory temperatures.

Wind test cage

The study animal was enticed into the small recovery/wind cage (2.4 x 1.2 x 1.5 m) where it was monitored under exposure to alternating periods of wind - no wind of four hour duration. Wind was provided by two industrial fans mounted for maximum exposure 1.2 m from the cage. Wind intensity, as determined with a totalizing anemometer (Casella Instr., London, England), was approximately 17.6 km/hr (4.9 m/sec). All observations were made from a heated booth located 1 m from the cage.

Respiration chamber

The respiration chamber used in this study was a metal cylinder 135 cm in diameter and 180 cm in length with a functional volume of approximately 2,300 liters. Openings for air intake and gas collection were installed on opposite ends of the chamber. An otherwise airtight seal was obtained by use of silicone and grease where necessary. Four weight-sensitive load cells (W.C. Dillon Co. Ltd., Van Nuys, Calif.) were placed into the system, one at each corner of a steel plate floor. Movements of the bear were determined by changes in millivolts, as expressed on a 120 VAC digital voltmeter (Weaton Instr. Inc., Newark, N.J.).

A centrifugal pump (Bodine Electric Co., Chicago, Ill.) pulled air past the bear and through a 5 cm diameter hose at a flow rate of approximately 200 l/min.

Treadmill

The treadmill used in this study was hydraulically powered and equipped with variable speed control. A steel chamber (2.8 x

1.3 x 1.1 m) enclosing the treadmill tract served as an open circuit respiration chamber (Øritsland 1976). Design modifications since 1976 are partially noted in Hurst et al, 1980 (in preparation). Modifications included improved chamber sealing by the application of silicone and plexiglass windows. A rubber seal was mounted on the treadmill to make contact with the movable tract. A removable plexiglass window could be attached and sealed with grease prior to each session. Two mixing pumps (Bodine Electric Co., Chicago, Ill.) with a ventilation rate each of 400 l/min ± were mounted externally. Circulation of internal air via attached hoses limited stratification of gases within the chamber.

Surgery

A new operating theatre was constructed in the months prior to the anticipated surgery. This 4.6 x 5.5 x 3.6 m structure was divided into a "scrub" room and an aseptic operating room. Sterile gowns, masks and gloves were used to limit infection of implantation sites during surgery. See Appendix 4.

3.1.3. Experimental Design and Measurements.

Sequence of experimentation

Three experimental animals were studied over a six week period; individual bears were phased through an essentially identical sequence of observations and measurements. Bear 1, as discussed earlier, was not used in the oil exposure study. The sequence of experimentation began with Bear 2, followed by Bear 4 and Bear 3 respectively. Methodology was standardized both within and between study animals, each animal acting as its own control.

Body temperatures (deep body and skin) were recorded throughout the test sequence. A schedule of events for each animal is presented in Figure 3-2 and Appendix 3 and followed the general design of:

1. Determination of resting metabolism (Respiration chamber - 3 days).
2. Activity metabolism (treadmill - 4 days).
3. Wind tests (wind test cage - 1 day).
4. Oil exposure (pool - 15 to 50 min.).
5. Wind tests (1 day).
6. Resting metabolism (3 days).
7. Activity metabolism (4 days).

Mechanical breakdown of the treadmill prevented the activity testing of Bear 4 and Bear 3 after oil exposure.

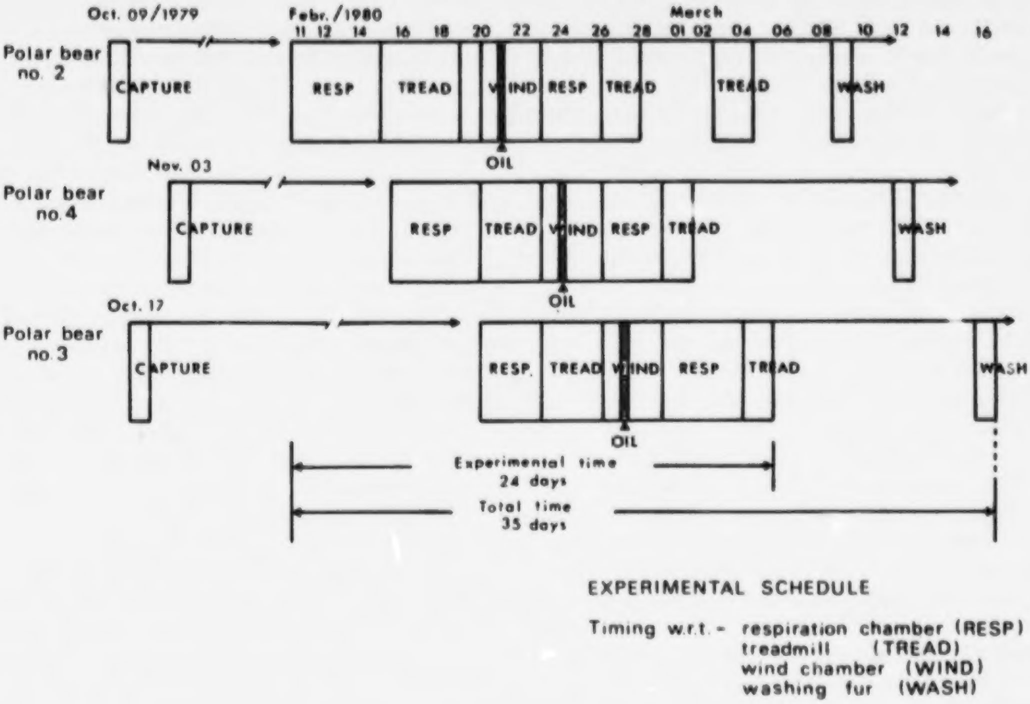

Oct. 09/1979
Febr./1980
March
11 12 14 16 18 20 22 24 26 28 01 02 04 06 08 10 12 14 16
Polar bear no. 2
CAPTURE
RESP
TREAD
WIND
RESP
TREAD
TREAD
WASH
OIL
Nov. 03
Polar bear no. 4
CAPTURE
RESP
TREAD
WIND
RESP
TREAD
WASH
OIL
Oct. 17
Polar bear no. 3
CAPTURE
RESP.
TREAD
WIND
RESP
TREAD
WASH
OIL
Experimental time 24 days
Total time 35 days
EXPERIMENTAL SCHEDULE
Timing w.r.t. - respiration chamber (RESP)
treadmill (TREAD)
wind chamber (WIND)
washing fur (WASH)

Figure 3-2. Schedule of experimentation for 3 oil-exposed polar bears.

Oil contact

Sea water (6,800 l or 1,800 gal.) was obtained from Hudson Bay in October 1979, and stored until February 1980 at which time it was pumped into the completed pool. Temperature probes incorporated into the pool at two depths allowed for continual monitoring and subsequent regulation of water temperature. It was maintained at 0°C ± 2°C throughout the water column. This approximate temperature could be expected year round in the Beaufort Sea, for example, where surface water temperatures average - 1.4°C in summer and - 1.7°C in winter (Weller 1976).

The oil used in this study was Midale Crude (See Chapter 5 for details of composition). It had been aged an indeterminate amount of time on arrival in Churchill 14 months prior to experimentation. A 45 gal (170 l) barrel of oil was opened one month prior to *in vivo* studies to obtain samples for use in the *in vitro* fur cleaning study (See Appendix 2). Oil from the same source was introduced to the pool surface in an amount necessary to create a 1 cm slick (57 l), 36 hours prior to the first bear's contact. While the actual surface thickness of an oil slick depends particularly on surface tension and specific gravity of the oil involved, Wadhams (1976) found that oils tested had a mean surface thickness value (H) of up to 1 cm. Following exposure, the remaining surface thickness was calculated and more oil added. This addition was to compensate for mechanical and weathering losses of the oil during and after individual exposure episodes.

Animals did not enter the pool voluntarily. The study bears were enticed from the recovery/wind cage through a series of sliding doors to the pool edge. Seal blubber was used as incentive. Each animal was allowed time to investigate the pool surface. When the animal had reached sufficiently far out from the pool edge, an entrance door was closed forcing the animal into the pool. The bears were retained for different amounts of time as listed here:

Bear 2:	15 min.
Bear 4:	30 min.
Bear 3:	50 min.

The rationale for varying immersion times was to correlate the thermal response to the actual immersion in the oil/ice water medium, including the expected episodic reliance on sub-cutaneous fat as a second insulative defence (Øritsland 1969). The initial exposure time was conservative (15 min.) and was later increased to simulate swimming in ice leads of different widths. Continuity of methology and subsequent replicative sample size was therefore somewhat sacrificed.

After a designated time, the entrance door was opened allowing the bear to exit the pool and re-enter the recovery/wind cage for continued observation. A separate booth located above and to one side of the pool gave a vantage for continuous observation during approach, immersion and exit from the pool.

3.1.4 Petroleum Characterization

Samples of Midale crude oil as used in this immersion study were analyzed in detail. A standardized methodology was used for all hydrocarbon assays, as described in Chapter 5.

3.1.5. Test for Weathering of Oil

Since no oil samples were available from the time of exposure of the bears to the 36 hour-old experimental oil slick, that is, of a weathered oil, a simulated weathering study was carried out in the laboratory. The experimental conditions were as follows. A 216 cm^2 slick of Midale crude oil, 1 cm thick, was placed on the surface of a flow-through water system, exchanging at a rate of 10.5 l/hr. The volume of water exchanged over 24 h corresponded closely with the volume of the column of water in the exposure pool under a 1 cm thick slick of the above area. Water temperature of the simulated weathering system was 6°C, air temperature 4°C. The cold room in which the test was carried out had a constant exchange of air by cooling units. Samples were taken from the test slick at 24 h and 48 h of air and water exposure, to be analyzed for hydrocarbon fractions by gas chromatography.

3.1.6 Oil Residues in Biological Samples

Tissues and bile were obtained from three bears which had not been experimentally oil-exposed and served as controls. Plasma, urine, and feces were taken at numerous intervals for four months preceding the oiling event from four bears (Nos. 1, 2, 3 and 4). Three bears (Nos. 2, 3 and 4) which were exposed to oil were sampled for one to five months following the exposure. Thus, the pattern and degree of uptake of oil could be determined from plasma, vomitus, stomach contents, and fecal samples; its distribution assessed in tissues; the excretion of oil from the bears was examined in fecal, bile, and urine samples. The two bears which died approximately one month after the oil exposure (Nos. 3 and 4) supplied samples analyzed for residual oil.

The following general technique was used for all biological samples. Five g of tissues, stomach contents or fecal samples, or 5 ml of plasma, bile, urine, or vomitus, were extracted with 7.0 ml hexane-dichloromethane (5/2). Extraction was carried out over 10 h by rotation in a sealed glass and teflon vessel, at 4°C in the dark. Fluids were extracted directly; tissues were extracted after homogenization in 2 volumes of double-distilled water with a Polytron homogenizer (at less than ultrasonic-generating speeds). The following tissues were analyzed - liver, kidney (cortex and medulla combined), skeletal muscle (gracilis or triceps brachii), heart muscle (ventricle), brain (cerebrum, cortex and medulla combined), spleen, subcutaneous fat, mesenteric fat, marrow (from proximal shaft of humerus), and lung. Fur was also sampled from one bear.

All samples taken for hydrocarbon assessment were stored in glass, or wrapped in foil and then sealed in Whirl-Paks. They were frozen and maintained at -20°C until analysis.

3.1.7. Clinical and Toxicological Assessment

3.1.7.1. Hematology and Plasma Biochemistry

Blood samples were taken from the bears for clinical hematology and plasma chemistry at capture and at scheduled intervals during their maintenance in the laboratory. Samples taken before the dosing period were analyzed to establish control values for the bears for all parameters. Both a normal overall range for the four bears, as well as individual comparative control ranges, were established. Blood samples were then taken after the dosing period at more frequent intervals; the specific times are indicated on the tables of results. Sampling of all bears at Churchill ended with the death of Bears 3 and 4 towards the end of March, or at transfer at this time of Bear 2 to the Assiniboine Zoo in Winnipeg, Manitoba, where treatment and recovery were effected. Further samples were obtained from Bear 2 in Winnipeg and the course of recovery was followed for the parameters assessed in this report until September, 1980.

Hematological and plasma chemistry analyses were carried out both by F.R. Engelhardt, to assess the toxicological effects of petroleum exposure in polar bears, and by F.A. Juck, for health monitoring purposes for the Polar Bear Care Committee (PBCC). Some overlap of assays occurred, serving as mutual checks on methodology. Details are found in Chapter 6.

3.1.7.2. Pathology

Examination of crude oil-related gross and histopathology was initiated in order to maximize data collection on the effects of crude oil on polar bears when two animals were unexpectedly lost as a result of oiling.

A full spectrum of tissues and body fluids were taken immediately post-mortem from Bears 3 and 4, and processed for pathological examination. All tissues were fixed in neutral buffered formalin. An extensive photographic record was kept. The findings are described in Chapter 7.

3.2. *In Vitro* Tests on Fur Samples

Fur samples from three polar bears were obtained through the cooperating agencies of the Government of the Northwest Territories and Manitoba Department of Renewable Resources. Bears 1 (NWT 87) and 2 (NWT 101) were one year old siblings shot in Clyde River NWT. on October 2, 1978. Bear 3 was a two year old bear which died in Churchill in October of the same year. Two 50 x 50 cm squares from each animal were taken from body areas as indicated in Figure 3-3, fleshed and frozen without any chemical treatment. The six samples were shipped to Oslo in the summer 1980 for use in fur conductance measurements.

The design characteristics of the wind tunnel and other analytical equipment used to test conductance in these fur samples are described in Chapter 4. The coating effects of three types of oil of different viscosities were examined.

3.3. Computer Model of Heat Balance

Model description.

The polar bear heat balance model is based on using Kleiber's law for calculating heat production and Meeh's formula to determine the size of the heat dissipating surfaces. Kleiber's law is valid only for adult mammals resting under thermoneutral conditions and only after digestion and absorbtion of food is completed. Therefore, the actual heat production used by the model is determined by multiplying the values produced by Kleiber's law with factors reflecting heat balance and exercise effects.

Further, in order to include the variables of air temperature, wind and solar radiation, the thermal physical characteristics of a plane fur sample are combined with the physiological laws of Kleiber and Meeh. The rationale for such a combination was presented in 1974 (Øritsland 1974) and recently expanded to form a dynamic heat balance model for harp seal pups (Pagophilus groenlandicus) (Øritsland and Ronald 1978). By this method the total heat production may be dissipated through the surface as determined by Meeh's formula. However, a differentiation of the total heat loss into the parallel avenues of evaporation and convection is recognized. Hence, by using only the thermal properties of the dorsal body trunk fur, and using parallel heat loss functions for respiratory evaporation and heat loss from the appendages, a complete heat

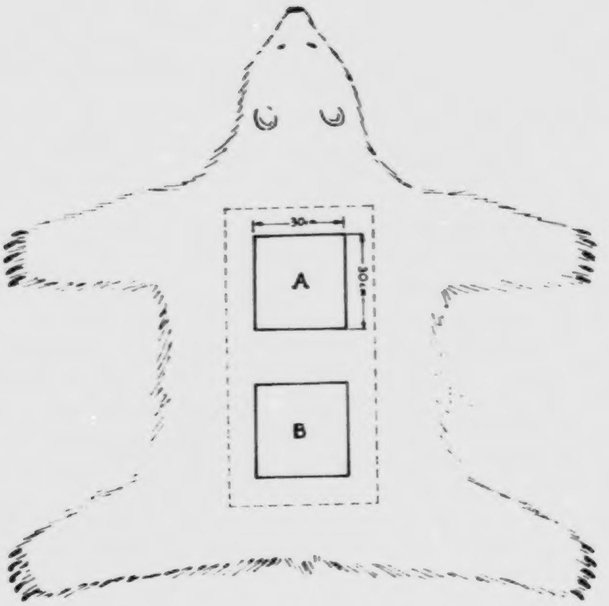


Figure 3-3. Polar bear fur:conductance/insulation study Positions from which pelt samples were taken.

balance model was established. The model was programmed in the computer language APL. A more detailed description of model design and performance is presented in Appendix 1.

4. THERMOREGULATORY EFFECTS

N.A. Øritsland, R.J. Hurst, and P.D. Watts

4.1. Introduction

Simplistically, the direct effect of oil on wildlife may be divided into toxicity and the physical change of an animal's defence against extreme ambient temperatures. The present work concerns the effects of crude oil on the heat balance of the polar bear, an animal naturally exposed to extreme environmental temperatures. It was considered that a study of the effect of oil on heat balance of warmblooded animals (homeotherms) had not yet been designed to examine and quantify changes in the physiological avenues of heat loss.

In the present laboratory work, measurements of metabolism, deep body temperature and skin temperature were performed on three polar bears both before and after oil exposure. Independent examinations were made on the effect of oil coating on the insulative value of fur samples. The results have been integrated by use of a mathematical model programmed in the computer language APL. The computer model is used both for interpretation of experimental results and to predict the effect of oil-exposure on free ranging polar bears.

4.2. Materials and Methods

4.2.1. *In Vivo* Studies

4.2.1.1. Temperature Measurements

Ambient air and water temperatures were monitored by use of standard meteorological recorders (Tempscribe clock monitor, Bachard Inst. Co., Pittsburg, Penn.). At the beginning of each experiment, the accuracy of Tempscribe temperatures was confirmed by use of thermistor probes (Model 401 A, Y.S.I., Yellowsprings, Ohio). Also, such thermistor probes were used to monitor respiration chamber air and floor temperatures.

Skin and deep body temperatures of the polar bears were determined by use of temperature sensitive radiotransmitters (J. Stuart Enterprises, Grass Valley, Calif.).

The transmitters were coated with paraffin to ensure water impermeability, resulting in a total size of 2 x 3 x 6 cm. Each had an expected life of 120 days according to the manufacturer. The expected life was overestimated as is demonstrated in the results. Transmitters used for body temperature measurements were calibrated for a range of 25 - 40°C. Skin transmitters were designed and calibrated for a range of 0 - 40°C. The output of each transmitter was recorded over the range of temperatures while immersed in a temperature controlled water bath. Period vs temperature regression equations were determined for each of the 7 transmitters used. The accuracy of the transmitters was within 0.1°C.

The temperature signals were received on one of two receivers (Model Tr-1-164-20, Telonics Telemetry and Electronics, Mesa, Arizona). Period was displayed on one of two digital processors (Telonics) and expressed in milliseconds. Transfixed omnidirectional antennas (Telonics) were used in most cases. Short distances of less than 10 m facilitated good reception of signals. A "whip" antenna mounted within the treadmill structure minimized interference from external fans, evacuation pump and treadmill engine during treadmill measurements.

4.2.1.2. Gas Analysis

The air flows through the respiration chamber and the treadmill chamber were established by means of centrifugal pumps and determined by use of a 350 l gasmeter (W.E. Collins Inc., Braintree, Mass.)

Oxygen concentrations were measured by use of a zirconium oxide oxygen analyzer (Applied Electronics Inc., Sunnyvale, Calif.), and barometric pressure by use of a standard diaphragm manometer (W.E. Collins).

4.2.1.3. Implantation of Transmitters

The temperature transmitters were implanted 12-19 days prior to oil-exposure measurements. Each animal received one deep body and one skin temperature transmitter. The deep body transmitters were placed centrally and extraperitoneally about 20 cm posterior to the sternum (on the belly side, just outside the body cavity). This technique (as compared to intraperitoneal implantation) offers the advantages of inhibiting migration of the transmitter within the body cavity, of affording easier retrieval at the termination of measurements, and of minimizing the chance of infection. The skin temperature transmitter was placed subcutaneously on the left side of the neck, about 15 cm from the spine and 30 cm anterior to the shoulder blades.

In addition, Bear 3 was implanted with a skin temperature transmitter on the dorsal right side of the rump about 20 cm below the spine and 30 cm anterior to the tail.

General guidelines regarding animal care and a more detailed treatment of surgical procedures are presented in Appendix 4.

4.2.1.4. Wind Test Cage

During the periods in the wind test cage, movements and behaviour of the polar bears were continuously observed, timed and noted. This included the animals' position, orientation with respect to wind, and all activity: grooming, rubbing, aggressiveness, body excretions, etc. Throughout the entire observation period, core and skin temperatures were recorded at 15 min. intervals. Food was withheld and snow was provided ad libitum. As mentioned above, observation continued from a separate booth during immersion in the pool. Observations continued on return to the recovery cage for 40 hours following oil-exposure. Total observation time was 68 hours.

4.2.1.5. Respiration Chamber

In the respiration chamber, sampling was of two-hour duration on each of three or four successive nights at 2300 - 0100 hours. Disturbance factors were minimal at this time, allowing the best approximation of natural resting/metabolic patterns. The circulation fan and evacuation pump were turned on 2 - 3 hours prior to each measurement. Flow rate was recorded at 30 minute intervals throughout the sample period, and results were averaged. Values in all cases were corrected to STP. Oxygen concentration of ambient air outside the chamber was recorded before and after each sample period, and results were averaged. Oxygen concentrations were recorded every minute throughout the two-hour period. Deep body and skin temperatures were recorded every 10 minutes. In this manner, a relative index of activity during metabolic measurements was possible. If the animal's activity was not minimal, i.e. the animal was not resting, the measurements were delayed until such a time as they became so. If any extreme amount of movement was noticed throughout the course of recorded measurement, results were discarded.

A reading from the weight-sensitive load cells (millivolts) was taken at approximately 2-minute intervals throughout the sample period to confirm the activity observations.

4.2.1.6. Treadmill

A period of time was allowed on the treadmill before experimentation to allow for metabolic recovery from the effects of drugging. Time also elapsed between each run to allow the bear to return to resting metabolism. No significant training period was necessary. Each animal was run as control for three or four days at no more than 2 runs/day. The total number of control runs were: Bear 2 - 8 runs, Bear 4 - 4 runs, Bear 3 - 5 runs. Oxygen consumption was not determined during all runs. All runs were of 30-minute duration at a walking speed of 1.5 m/s (5.4 km/hr). Air flow rates were determined at 10-minute intervals throughout the sample period. Results were averaged. Oxygen concentration of ambient air outside the treadmill chamber was recorded before and after each experimental run, and results were averaged. Percent oxygen was recorded at 30-second intervals throughout the run and for 30 minutes following termination of the run.

Core and skin temperatures were recorded at 5 minute intervals beginning 20 minutes before the run commenced and proceeding for 70 minutes after the treadmill was stopped. Behaviour and position of the animal were recorded before and throughout each sample period.

4.2.1.7. Statistical Treatment

Statistical significance in all cases was tested at the 0.05 level of significance. 'T' tests were employed, computing 'T' for an unpaired series of samples from two populations, and did not assume equality of variance. Refer to Snedecor and Cox, 1967; pp 114 - 116. Regressions of fur conductance with wind (section 3.2.1) were obtained, again with use of the APL computer language, by determining the best fit of a line by multiple linear regression.

4.2.1.8. Rump Temperatures

The lateral neck site proved an appropriate location since it did not come in contact with a metal surface regardless of the animal's position. It became apparent during experimentation, however, that the rump temperature readings were markedly affected by the position of the animal relative to the cage floor; i.e. contact of the rump area with the steel plate would cause a rapid drop in observed rump skin temperature. Because of this 'artefact' the rump temperature measurements have not been included, except for those from treadmill trials, when the rump was assuredly free from contact with any structure. The skin transmitters in the neck of each bear were free from any such bias.

4.2.2 Fur Samples.

4.2.2.1. Wind Tunnel

General design, analytical equipment

The wind tunnel facility, housed in a greenhouse, is diagrammatically presented in Figures 4-1 and 4-2.

Fur samples measuring 30 x 30 cm were mounted over a heat flow disc (Øritsland and Smith 1975, Øritsland and Lavigne 1976). The disc, imbedded in a layer of grease, was positioned over a steel chamber through which water was continuously circulated. Water temperature was maintained at 38°C ± 0.2°C by thermostat control. Layers of grease between the fur sample and steel chamber assured uniform thermal contact.

A thermoelement inserted from the underside of the skin allowed measurement of skin surface temperature. A second thermoelement was positioned below and to the side of the fur sample, shaded from sunlight, to measure air temperature. Temperatures were expressed on a digital thermocouple thermometer (Digitec United Systems Corp.) and heat flow on a D.C. millivoltmeter (Digitec). Solar irradiance was measured with an Eppley Solarimeter mounted parallel to the fur surface and recorded on a compensating recorder.

The fur sample was positioned at an angle with respect to wind direction in order to simulate the spherical nature of an animal's body (Larose 1947, Lentz and Hart 1960). Winds up to 4 m/sec. were generated using a variable speed fan to pull air across the fur by suction rather than directing air towards the fur (Øritsland 1974, Øritsland and Smith 1975). Wind speeds were measured with a hot wire anemometer (Wallac Oy, Turker, Finland) clamped 10 cm above and parallel to the fur surface.

A series of preliminary tests was designed to quantify the effect of alteration in tunnel design on turbulence. A lattice of cardboard tubes positioned to intercept air before reaching the fur (Figure 4-1) reduced turbulence and variations in wind speed. One suction fan (fan 1) in combination with this lattice of tubes, produced the most uniform wind profile and was subsequently used in all fur conductance experimentation (See Figure 4-2).

4.2.2.2. Theoretical Considerations

The amount of heat flow through a fur is determined by the physical nature of the fur, and the temperature gradient, in the following manner:

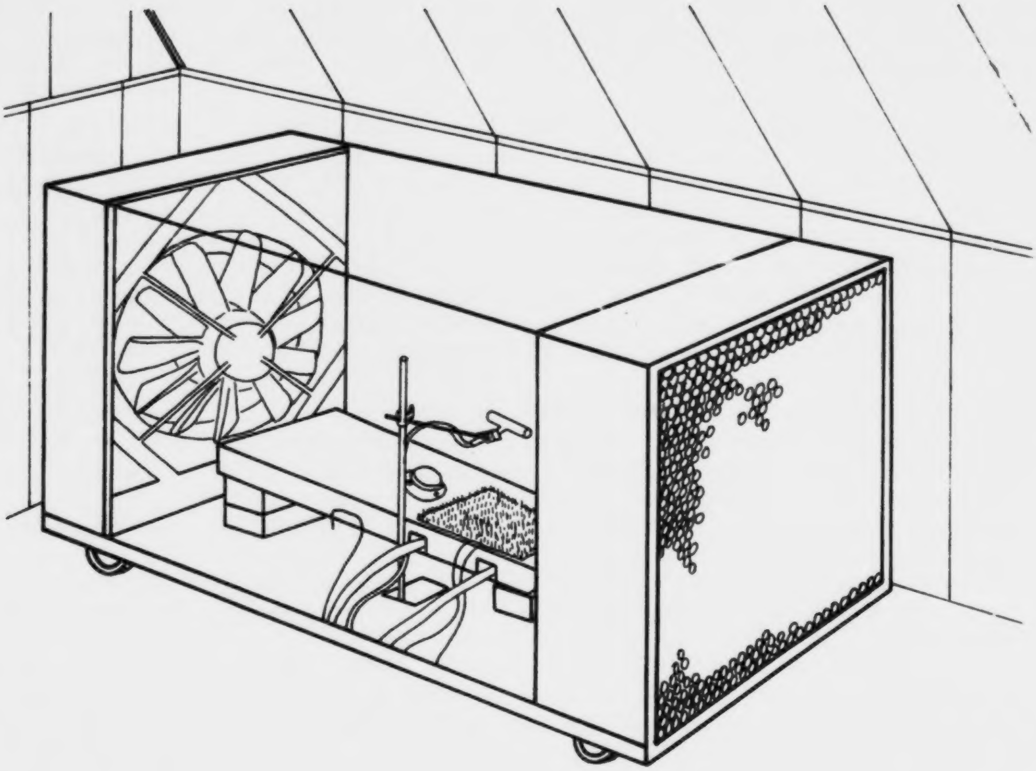
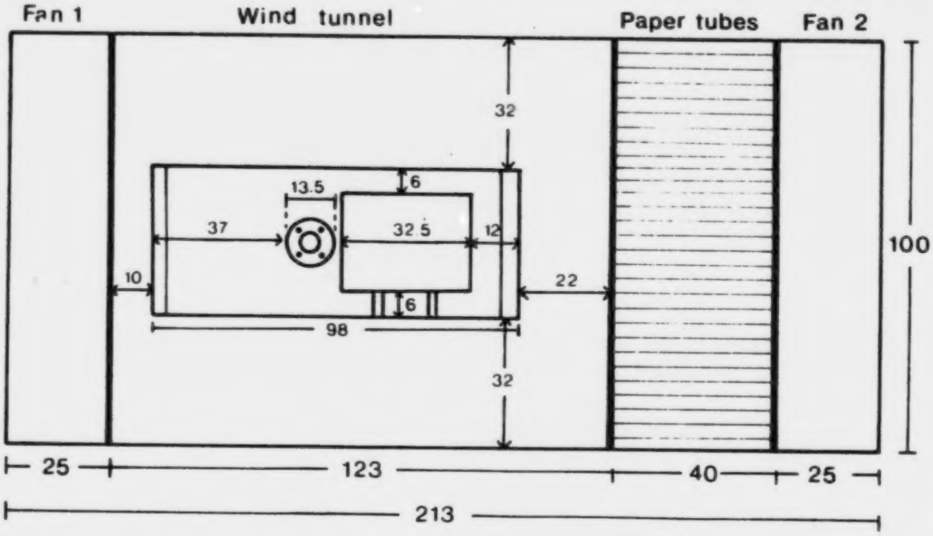
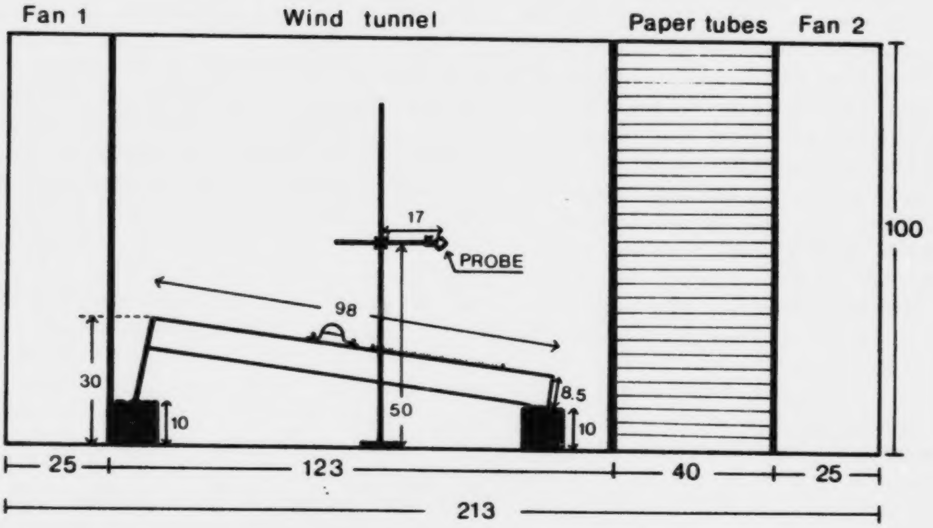


Figure 4-1. Wind tunnel used in the polar bear fur conductance/insulation measurements. The tunnel itself is enclosed in a greenhouse.


Fan 1
Wind tunnel
Paper tubes
Fan 2
32
13.5
6
37
32.5
12
10
22
6
98
32
100
25
123
40
25
213

Top view


Fan 1
Wind tunnel
Paper tubes
Fan 2
17
PROBE
98
30
10
50
8.5
10
100
25
123
40
25
213

Side view

Figure 4-2. Wind tunnel used in the fur sample conductance/insulation study.

$$HF = h_c(T_s - T_a)$$

where

HF = heat flow (W/m^2)

h_c = convective heat transfer coefficient ($W/m^2/°C$) characteristic for the fur

T_s = skin surface temperature (°C)

T_a = air temperature (°C).

Fur conductance has been shown to be a function of wind speed as well (Lentz and Hart 1960, Treager 1965), such that

$$h_c = h + fV^b$$

where

h = fur conductance in calm air ($W/m^2/°C$)

V = wind speed (m/sec)

f and b are experimentally determined factors.

This experiment was designed to determine the conductance of polar bear fur as a function of wind speed, before and after physical fouling with oil.

Solar radiation acts to reduce the amount of heat flow from the skin to the environment or to generate heat flow towards the body. Heat load at skin level per unit of solar irradiance on a fur sample may be expressed as a factor (U) called the solar utilization factor (Øritsland 1973, Øritsland 1974).

To determine U, the difference in heat flow with and without solar radiation is experimentally determined such that:

$$HF = HFT - HFS$$

where

HF = difference in heat flow attributable to solar radiation (W/m^2)

HFT = measured heat flow in the absence of solar radiation (W/m^2)

HFS = measured heat flow with solar radiation (W/m^2).

Solar utilizatiion of the fur (U) is then expressed as a factor of solar radiation such that:

$$U = \frac{HF}{SR}$$

where

SR = measured incoming solar radiation (W/m^2).

4.2.2.3 Oil Properties and Exposure

Three types of oil were investigated. Rationale in selecting these oils was to test the effect of oil of different viscosities on the thermal properties of polar bear fur.

Several series of measurements were performed on two separate pelts using samples of the same Midale crude oil used for the live animal study in Churchill (see Chapter 5 for characteristics). Two other types of oil were also utilized: 10 W Motor Oil (Shell), and Stanco Base 2500 (Norske Esso). The viscosity of the three oils with respect to temperature are presented in Figure 4-3.

Control values of the heat transfer coefficient were determined over a period of days for each of four fur samples. Measurements were first taken with the wind chamber in darkness. Several recordings of heat flow and temperature gradient were made at each of four wind speeds. A time period of about 30 minutes between each measurement was allowed for stabilization of temperatures and heat flow.

The wind tunnel was then uncovered and the fur exposed to direct sunlight. A continuous recording of solar irradiance permitted accurate estimation of continuity as well as amount of sunlight. A series of measurements was made in a method similar to that performed in darkness and a solar utilization factor was calculated.

At the completion of control measurements the fur sample was coated with oil and several series of measurements were repeated in the aforementioned manner to determine the change in U and h_c. The application of oil was achieved by immersing the fur in a water bath, covered with a 1 cm slick of oil (1 litre) as *in vivo* (see Chapter 3). Water, oil and fur were stable at ambient temperatures of 15°C to 20°C. The fur was agitated in the water-oil mixture for 5 minutes and allowed to 'soak' for another 25 minutes. Care was taken to avoid oil contact with the skin side of the pelt.

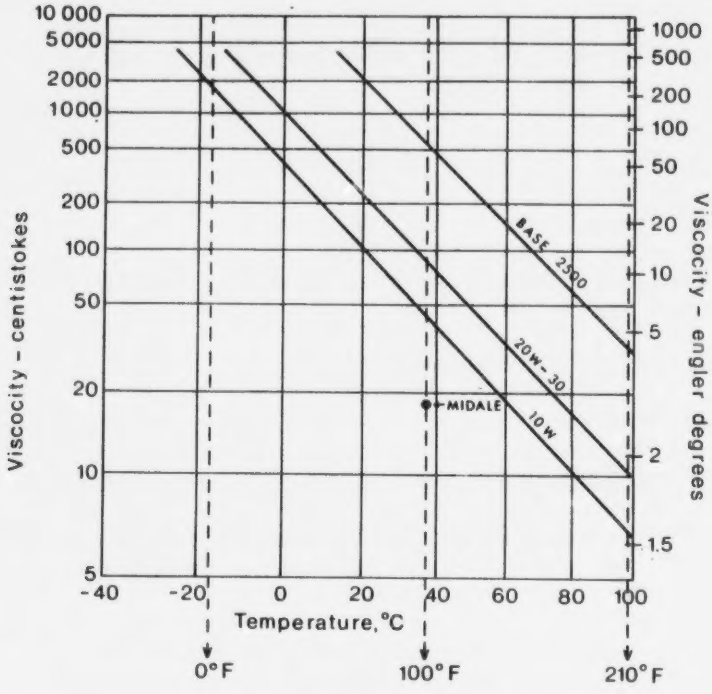


Figure 4-3. Viscosity-Temperature diagram. Oils used in the fur conductance study were Base 2500 (Norske Esso), 10 W motor oil (Esso) and Midale crude (Saskatchewan). Viscosity is given in both centistokes and engler degrees.

4.3 Results

4.3.1. *In Vivo* Studies

4.3.1.1 Oil contact

All animals reacted to enclosure in the unfamiliar pool complex in an active, aggressive manner. While in the water, the bears' behaviour alternated between attempts to escape and attempts to reach the inaccessible seal blubber offered as bait. All animals used their forepaws to grip the vertical cage bars for support and to encircle the pool confines, and made few attempts to actually swim. All animals left the pool immediately when the ramp door was re-opened.

Oil adhered rapidly to fur which came in contact with the oil. The actual amount of oil on the fur of each animal was difficult to quantify; however, several indirect means of estimation were possible.

Following each of the first two trials, the thickness of the remaining surface slick was measured. The calculated discrepancies in volume correspond to a loss of 23 and 28 liters of oil after immersion of Bears 2 and 4 respectively. Each animal was weighed pre-oil exposure and two and five (or seven) days post-oil exposure (see Appendix 3). Weight increments from pre- to two days post-oil were 9, 21 and 27 kg for Bears 2, 3 and 4 respectively.

At this time they were placed in the respiration chamber to which a quantity of snow and sawdust had been added. On emergence three days later the animals were visibly much cleaner and a large amount of oil remained mixed with the sawdust. This amount was calculated as 20 kg following emergence of Bear 3. Weight decrements of the animals during the three days in the respiration chamber were 14, 25 and 24 kg for animals 2, 3 and 4 respectively.

4.3.1.2. Wind Tests/Behaviour

Behaviour - qualitative description.

All animals were habituated to cages and so showed little difficulty in adjusting to the wind/observation cage. The animals were occasionally aggressive but most time was spent in sleeping or resting. When subjected to wind the bears were more inclined to sleep or assume a metabolically efficient body posture. The curled position as described in Øritsland (1970) was observed more frequently during wind exposure both before and after oiling.

The cage structure was cleaned between trials. However. enough oil residue remained on the plate and bars to cause minor contamination of the animals' paws. The 2nd and 3rd bears to enter the cage (Bears 4 and 3 respectively) subsequently spent considerable time grooming as well as searching for oil and licking it from the structure. Such behaviour was not observed for Bear 2 which entered a clean cage.

Following oil exposure in the pool, the bears acted aggressively on reentering the wind cage. They commenced licking the oil from their fur almost immediately and groomed actively for several hours. The licking was concentrated on the paws and forelegs. This behaviour was interrupted frequently by the animal forcefully shaking its body. Bear 4 was the only animal observed to rub to any extent on the snow or cage bars. This bear often circled the cage confines, violently rubbing its fur. All bears were observed licking oil from the steel floor as well as from their fur.

All bears appeared reluctant to lie on the oil-covered steel floor and occasionally seemed to sleep while sitting or standing supported by the cage wall.

Shivering was not noted in any of the pre-oil observations. The onset of shivering in all bears occurred within 8 hours of oil exposure and subsequent ingestion (Bear 2 - 8 hours, Bear 3 - 5 hours, Bear 4 - 5 hours). Intensity of shivering oscillated with time and was most pronounced in Bear 4, least pronounced in Bear 2.

All bears vomited on more than one occasion beginning within 12 hours of the onset of oil ingestion. They also exhibited a diarrhetic response; both vomit and faeces showing high oil content (see Chapter 5). Snow intake increased substantially immediately following oil ingestion.

Behaviour - quantification

An attempt has been made to express behavior quantitatively in the following manner:

Activity level coefficients were assigned according to the principle of Wunder (1975) and Taylor et al. (1970) and as used for polar bears by Best (1976). In this study, metabolic activity levels were arbitrarily assigned values ranging from 1.0 for a sleeping, lying animal to 2.2 for an extremely active, aggressive animal.

For each bear, 68 hours of observation are expressed by an activity level for each of 272 15-minute time intervals.

Grooming activity was expressed according to absolute time in minutes, and percent time spent grooming. A chronological record of each 68-hour period is expressed graphically in Figure 4-4. Analysis included comparisons between: individual animals Before Oil Exposure vs. After Oil Exposure, and No Wind vs. Wind.

When subjected to wind all bears exhibited a lower mean activity level than during periods of no wind. Overall activity values for Wind vs. No Wind are:

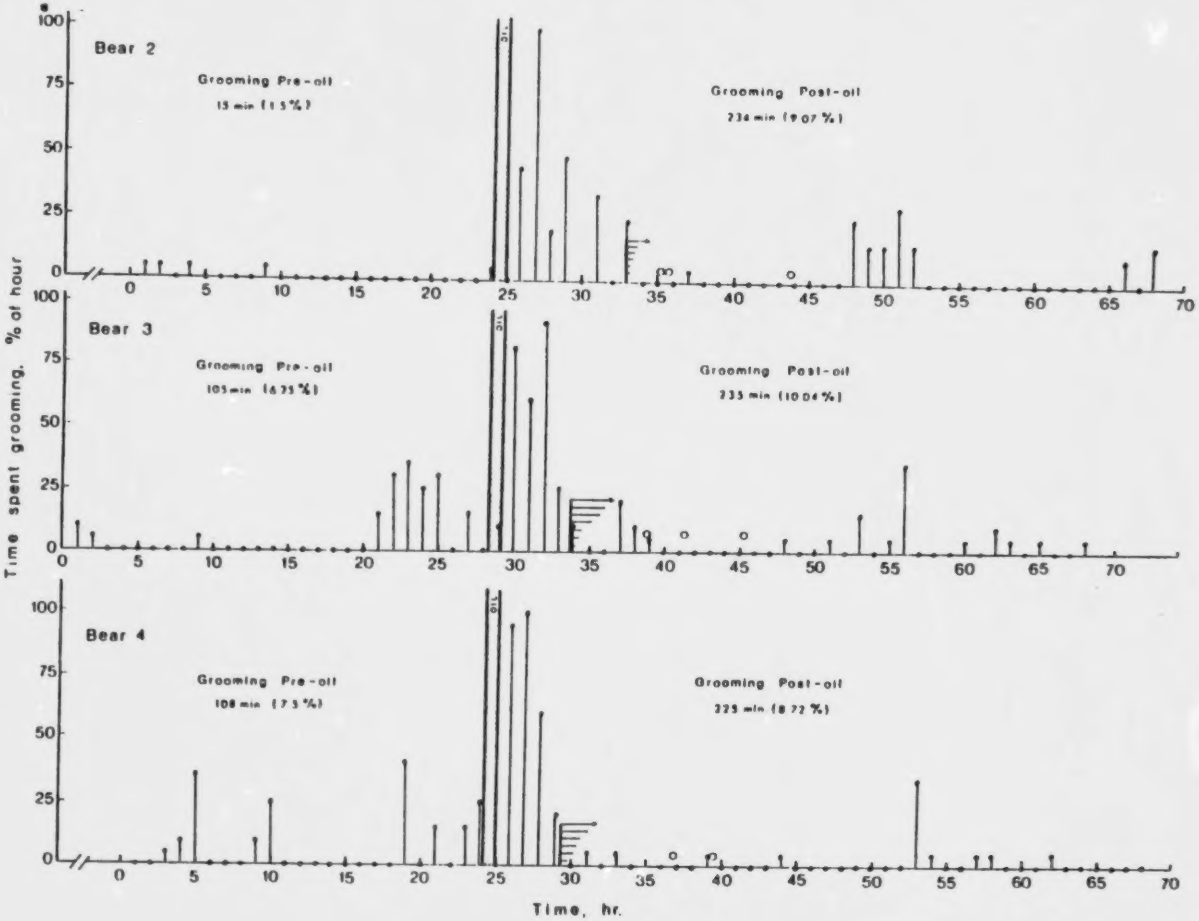


Figure 4-4. Grooming behaviour of three study animals throughout the 68 hour observation period. Histogram - vertical lines represent percent of time per hour engaged in licking. Horizontal lines indicate onset of shivering which continued through the duration of the observation period. Open circles designate times of vomiting.

	Wind	No Wind
Bear 2	1.13 ± 0.2	1.28 ± 0.34
3	1.40 ± 0.35	1.44 ± 0.36
4	1.19 ± 0.27	1.33 ± 0.35
Overall Average	1.24 ± 0.31	1.35 ± 0.35

Analysis of Pre vs. Post-Oil activity levels shows an increment following oil, as follows:

	Before Oil	After Oil
Bear 2	1.15 ± 0.27	1.25 ± 0.30
3	1.30 ± 0.39	1.51 ± 0.31
4	1.28 ± 0.39	1.25 ± 0.27
Overall Average	1.25 ± 0.36	1.33 ± 0.32

A comparison of activity level before and after oiling is masked by high activity in the hours immediately after oil contamination followed by a general listlessness in the later obervations. It is further influenced by the animals occasionally sleeping while standing or sitting following oil contamination. High values for standing or sitting were thus assigned, rather than low values for sleeping. The above higher activity following oil is consequently not an accurate reflection of real activity.

Grooming

The first animal to enter the wind chamber spent little time grooming prior to oil exposure (15 min. or 1.0%). Bears 3 and 4 spent a significantly greater amount of time than Bear 2: 105 min (6.3%) and 108 min. (7.5%) for Bears 3 and 4 respectively.

The time engaged in grooming following oil contamination was remarkably consistent among the bears, at 234 (9.1%), 235 (10%) and 225 (8.7%) minutes for Bears 2, 3 and 4 respectively.

Licking activity was concentrated in the time immediately after exit from the pool; comprising 35, 46 and 46% of the first 6 hours and 23, 26 and 26% of the first 12 hours for animals 2, 3 and 4 respectively.

Temperatures

Ambient temperatures (°C) ranged from - 16 to - 24°C during the observation periods.

Bears 2 and 4 showed an insignificant change in mean deep body temperatures (°C) soon after oil exposure, with before and after values of 36.9 ± 0.2 vs. 36.87 ± 0.5 (Bear 2)and 36.6 ± 0.3 vs 36.7 ± 0.4 (Bear 4). Bear 3 exhibited a significant decrement in deep body temperature in the last hours of observation resulting in mean values of 36.8 ± 0.3 vs. 35.8 ± 1.1 for Pre- vs. Post Oil.

Standard deviations were higher in all cases following oiling, an observation reflected in the oscillation apparent in the Deep Body Temp. vs. Time graphs (Figure 4-5).

Mean deep body temperatures during wind exposure were slightly lower than in non-wind periods for each study animal in the following manner:

	Wind	No Wind
Bear 2	36.87 ± 0.5	36.88 ± 0.3
3	36.05 ± 0.1	36.43 ± 0.8
4	36.67 ± 0.3	36.71 ± 0.4
Group Average	36.52 ± 0.8	36.68 ± 0.6

Skin temperature showed an average increment of 0.96°C after oil fouling. The skin temperature rise was consistently significant in all animals as follows:

	Before	After
Bear 2	36.4 ± 0.69	37.3 ± 0.43
3	34.9 ± 1.16	35.7 ± 0.43
4	32.9 ± 2.09	34.0 ± 1.81
Group Average	34.76 ± 2.0	35.72 ± 1.7

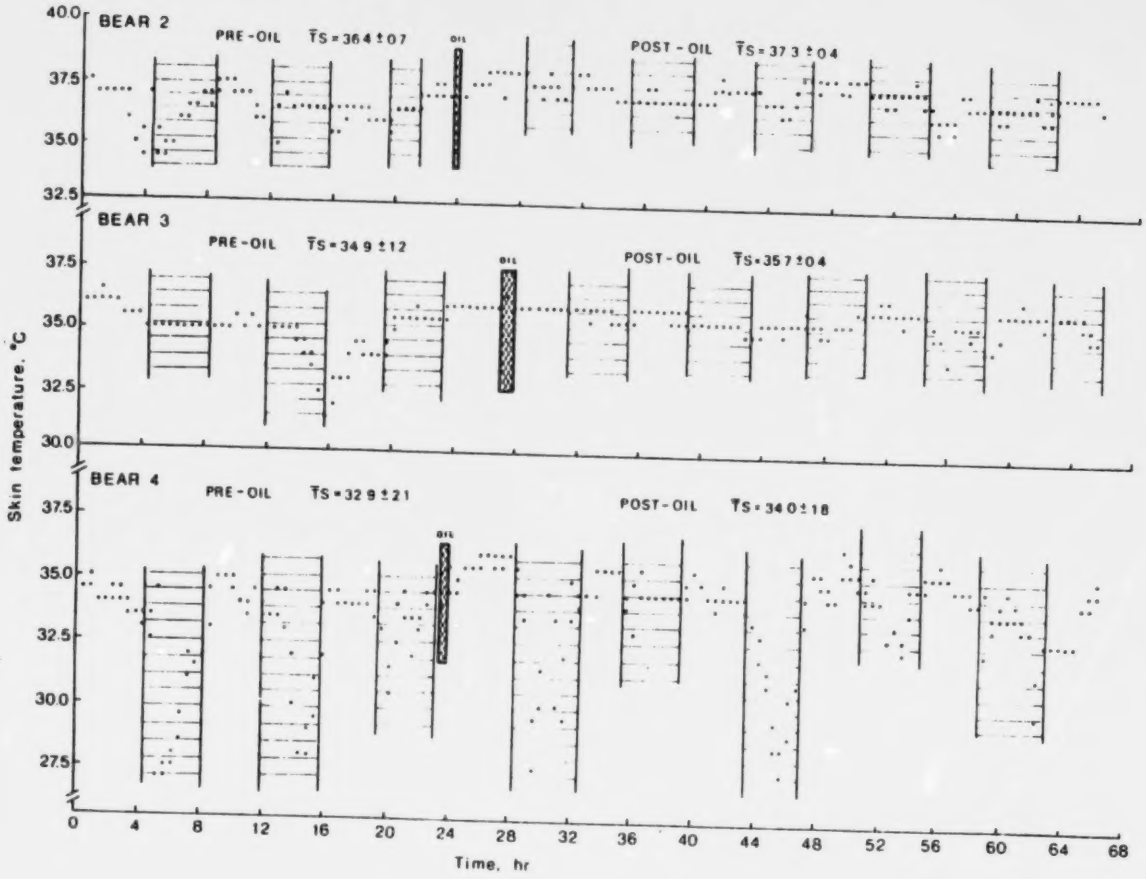


Figure 4-5. Wind chamber results illustrating skin temperature fluctuations over 68 hours, including pre- and post-oil observations. Means ($\overline{TS}$) and standard deviations (±) are included for both before and after oiling. Hatched areas represent periods of wind exposure (5 m/sec) and open areas are periods of no wind. Dots show skin temperatures at 15 min. intervals i.e. total of 272 intervals.

Mean skin temperatures were likewise significantly higher in periods of No Wind vs. Wind:

	Wind	No Wind
Bear 2	36.8 ± 0.66	37.1 ± 0.70
3	35.3 ± 0.78	35.5 ± 0.87
4	32.5 ± 2.2	34.7 ± 0.7
Group Average	34.79 ± 2.3	35.88 ± 1.3

Bear 4 showed the most dramatic response in skin temperature to wind as expressed in graph. (Figure 4-6).

4.3.1.3. Respiration Chamber/Resting Polar Bears

Metabolism

Oxygen consumption, as determined from 10 days pre-oil and 9 days post-oil measurements, clearly increased following oil exposure. Mean daily (25 measurements per day) and minimum daily values of metabolic rate expressed as a fraction of expected basal are presented in Figure 4-12.

Pre-oil values of metabolic rate were in general agreement with expected values as predicted by Kleiber's formula of basal metabolic rate (BMR = 3.4 $W^{3/4}$). The post-oil metabolic levels were, however, consistently higher than predicted values (See Figure 4-7).

Expressed as a fraction of predicted metabolism (Kleiber's law), mean minimum daily metabolism increased from 0.93 ± 0.20 to 1.38 ± 0.03 in Bear 2; 1.37 ± 0.03 to 1.70 ± 0.19 in Bear 3; 1.11 ± 0.07 to 2.05 ± 0.10 in Bear 4 following oiling. This represents a significant mean increase for all animals of 93 Watts or 64%.

Air temperatures in the respiration chamber ranged from - 16°C to + 3°C and no correlation between metabolism and chamber ambient or laboratory ambient air temperature was evident in the data.

Temperatures

Deep body temperatures of control animals were in good agreement with values obtained from polar bears in previous studies (Best et al. 1976, Øritsland 1970), averaging 0.4°C lower than values derived from intraperitoneal transmitter implantations.

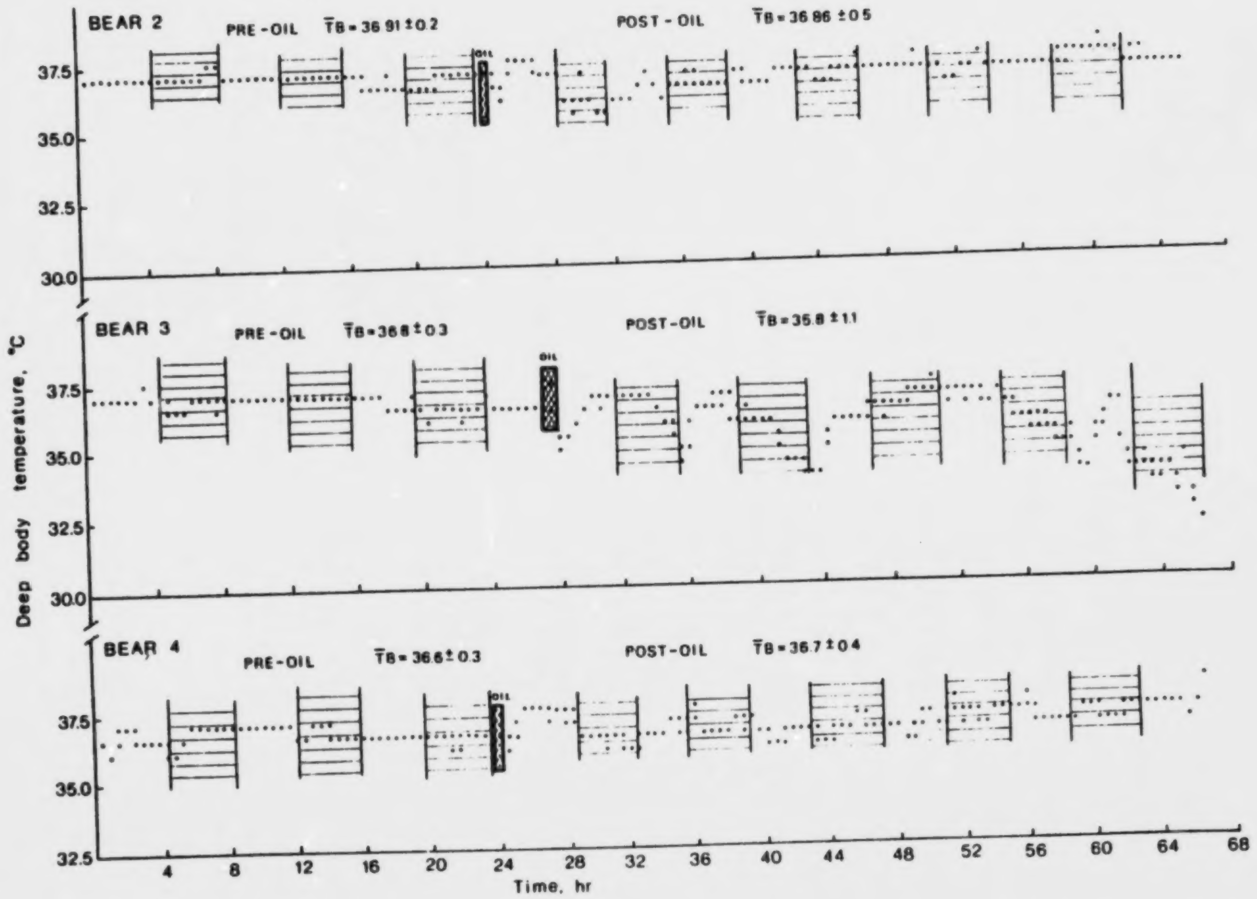


Figure 4-6. Wind chamber results illustrating deep body temperature fluctuations over 68 hours, including pre- and post-oil observations. Mean (TB) over 68 hours, including pre- and post-oil observations. Mean (TB) and standard deviations ($\pm$) are included for both before and after oiling. Hatched areas represent period of wind exposure (5 m/sec) and open areas are periods of no wind. Dots show deep body temperatures at 15 min. intervals i.e. total of 272 intervals.

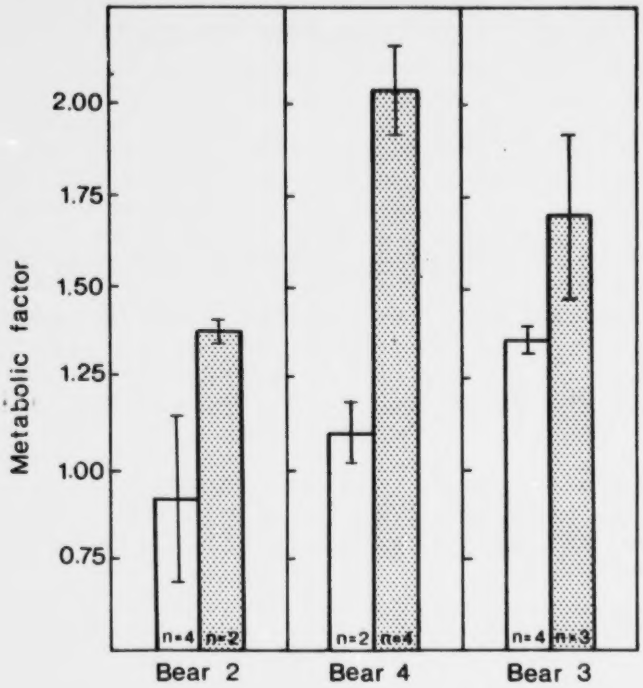


Figure 4-7. Experimental metabolic rate expressed as a factor of predicted basal metabolic rate. Histograms are average of minimum daily metabolic rate; n is number of days (25 measurements/day). Open rectangles represent values from pre-oil polar bears, hatched rectangles, post-oil polar bears. Vertical lines (I) show standard deviations of means.

Deep body temperature increased from 36.5 ± 0.2°C before oiling to 36.9 ± 0.1°C after oiling, and 35.8 ± 0.8°C before oiling to 36.6 ± 0.6°C after oiling in Bears 2 and 4 respectively. Polar Bear 3, however, exhibited a decrement in deep body temperature from 36.8 ± 0.6°C before oiling to 36.1 ± 0.9°C after oiling.

Skin temperatures increased significantly in all animals after oil exposure. In Polar Bear 2 skin temperature shifted from 35.5 ± to 0.3°C to 37.3 ± 0.2°C; in Bear 3, from 36.3 ± 0.1°C to 36.7 ± 0.2°C; and in Bear 4, from 34.5 ± 1.5°C to 35.7 ± 0.7°C.

There appeared to be no correlation between body temperatures (both deep body and skin) and the ambient or air chamber temperature.

4.3.1.4 Treadmill/Exercising Polar Bears

Metabolism

Comparative results of exercise, pre- and post-oil exposure were obtained for Bear 2 only (Figures 4-17 and 4-18). Mean oxygen consumption, as measured in the final 12 minutes of each run, were 1.68 ± 0.4 ml/g/hr pre-oil vs. 2.10 ± 0.2 ml/g/hr post-oil. This constitutes a mean elevation in metabolic rate of 262 watts following oil exposure (1416 ± 32 W pre-oil vs. 1678 ± 151 W post-oil).

Control values of mean oxygen consumption for Bears 3 and 4 were 1.77 ± 0.1 ml/g/hr and 2.11 ± 0.1 ml/g/hr, constituting mean metabolic rates of 1581 ± 116 W and 1281 ± 61 W respectively.

Expressed as a factor of basal metabolic rate according to Kleiber, Bear 2 exhibited a change from 9.5 ± 0.2 to 11.8 ± 0.9, a 24% increase following oil exposure. Control values of metabolic factors were 10.3 ± 0.8 and 10.8 ± 0.5 for Bears 3 and 4 respectively.

Temperatures

The mean difference between pre- and post-oiling deep body temperature was 0.33°C as measured prior to each run (36.51 ± 0.4 pre-oil vs. 36.84 ± 0.61 post-oil). At the end of runs, the pre- and post-oil deep body temperature difference was more substantial, averaging 0.88°C (37.85 ± 0.26 pre- vs. 38.73 ± 0.54 post-oil). Thus, the deep body temperature increment as a result of 30 minutes running was greater following oil exposure (1.35°C pre-oil vs. 1.89°C post-oil) (see Figure 4-18).

In control measurements Bears 3 and 4 had mean deep body temperature prior to running of 37.36 ± 0.48°C and 36.0 ± 0.46°C respectively. The maximum deep body temperatures following running were 38.87 ± 0.37 and 38.18 ± 0.98 respectively. Thus increments as a result of running were 1.5 ± 0.23°C and 2.18 ± 0.71°C for Bears 3 and 4.

The skin temperature transmitter in Bear 2 ceased to function after the first post-oil treadmill measurement. However, the difference between mean pre-oil and the one post-oil skin temperature as measured prior to each run was substantial at 0.9°C (36.92 ± 0.27 pre-oil vs. 37.82 post-oil). At the end of the run the post-oil skin temperature was marginally higher (0.2°C) than the pre-oil skin temperature (37.8 ± 0.15 pre-oil vs. 38 post-oil). Thus the skin temperature increment as a result of running was much smaller following oil exposure (0.88 ± 0.29 pre- vs. 0.18 post-oil).

Control measurements of Bears 3 and 4 showed skin temperatures prior to running of 36.19 ± 0.49°C and 34.53 ± 0.22°C respectively. Mean maximum skin temperatures following a 30-minute run were 37.97 ± 0.39 and 36.38 ± 0.73. Skin temperature increments as a result of running, therefore, were 1.78 ± 0.23°C and 1.85 ± 0.59°C for Bears 3 and 4 respectively. The rump temperature of Bear 3 increased an average of 3.0 ± 1.4°C from a mean pre-run temperature of 34.9 ± 1.6°C to a mean maximum temperature of 37.9 ± 0.3°C.

No correlation was apparent between body temperatures and ambient temperature which ranged from - 12 to - 27°C throughout the trials.

4.3.2 Experiments on Fur Samples

4.3.2.1. Conductance/Insulation

Experimentally determined conductance equations for four control (non-oiled) furs expressed as a function of wind speed were as follows:

$$\begin{aligned} h_c\ 1A &= 1.95 + 0.13\ V \\ h_c\ 2A &= 1.77 + 0.13\ V \\ h_c\ 3A &= 1.86 + 0.02\ V \\ h_c\ 1B &= 1.42 + 0.17\ V \end{aligned}$$

Average value of the 4 pelts was 1.75 (± 0.2) + 0.11 (± 0.06) V where

h_c = fur conductance (W/m^2/°C)
V = wind speed (m/sec).

Immersion of the fur in oil-water caused a substantial shift down in insulative value as expressed by a rise in fur conductance (h_c). Calm air conductance values (h) in relation to time of oiling are presented in Figure 4-8. Such values obtained the day of oiling (day 1) were 10.4, 8.9 and 9.0 $W/m^2/°C$ for pelts treated with Midale, 10 W and Base 2500 respectively. Due to the presence of water and the unstable nature of the oiled fur on the day of oiling, conductance values are presented using data only from the 2nd and 3rd days after oil exposure.

Linear regression of fur conductance on wind speed yielded the following equations (average of day 2 + day 3).

$h_c = 5.1 + 0.39$ V (Midale oil)
$h_c = 4.25 + 0.55$ V (10 W oil)
$hc = 6.17 + 0.74$ V (Base 2500 oil).

(See Figure 4-9).

The change in the insulative value of the pelts as a result of oiling are expressed by calm air conduction shifts (h - oiled, vs. h - non-oiled) of 275% for Midale oil, 240% for 10 W oil and 435% for the more viscous base 2500 oil. The wind coefficient, expressing the effect of wind on fur conductance, also increased, from an average of 0.11 pre-oil to 0.56 post-oil.

The influence of time on fur conductance was less significant for the viscous base 2500 oil, i.e. the h_c values showed comparatively little change throughout the three days of experimentation (See Figure 4-8). A fourth pelt, immersed in Midale crude oil was immediately exposed to sunlight. This caused a rapid 'melt' of oil from the fur surface. Analysis of the data obtained in the 2nd and 3rd days from oiling, carried out in conditions of no solar radiation, revealed a lower value than the aforementioned Midale oil equation (Figure 4-9).

$$h_c = 4.43 + 0.3 V.$$

4.3.2.2. Solar Utilization

Solar utilization (U) expressed as a fraction of incoming solar radiation was determined for three control (non-oiled) pelts. Calculated values were 0.102, 0.122 and 0.121 for furs 1A, 2A and 3A respectively.

Solar utilization increased in all cases following oil coating, with determined values of 0.145, 0.154 and 0.138 after application of Midale, 10 W and again Midale oils respectively. U was not determined using the Base 2500 oil treated pelt.

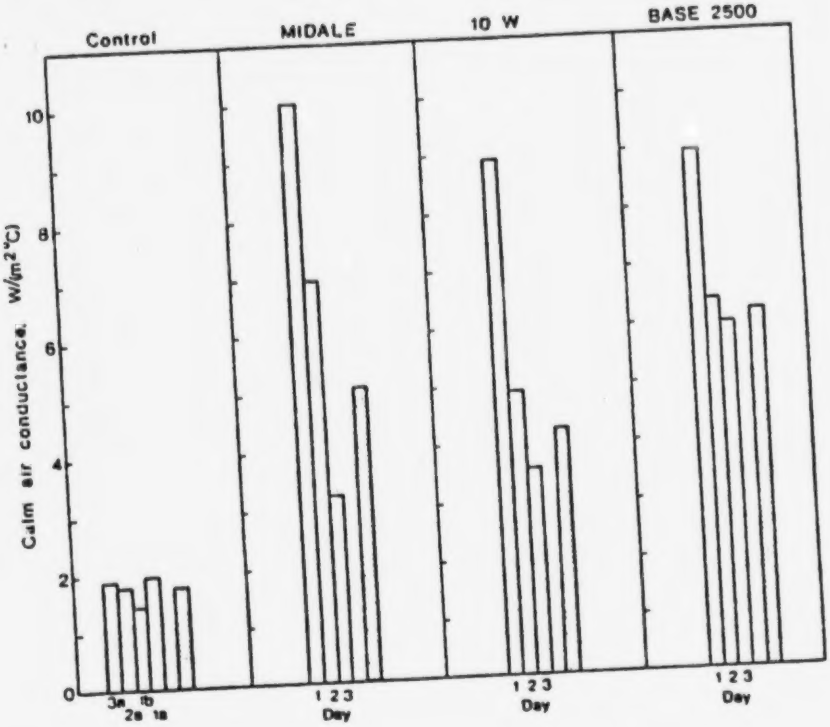


Figure 4-8. Calm air conductance values determined from 4 normal polar bear furs (control) and from furs following exposure to 3 types of oil. Values are presented for the 3 days following the day of oiling (day 1) and are represented by open rectangles, followed by the average of days 2 and 3 (closed rectangles). The shaded rectangle in "control" is the mean of 4 normal furs.

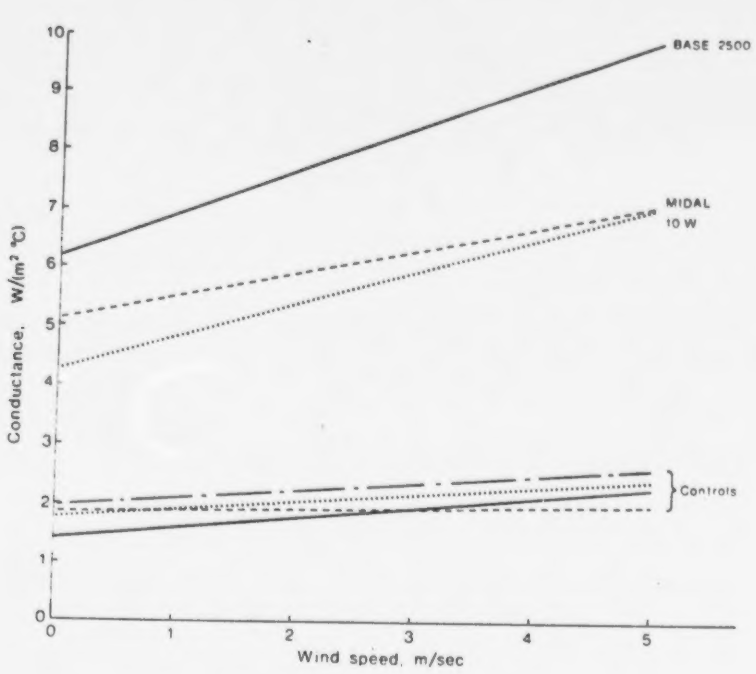


Figure 4-9. Fur conductance as a function of wind speed. Control values are derived from 4 pelt samples tested prior to oil contact. Furs were exposed to 3 oils: Midale crude, 10W motor oil and Base 2500 oil. All oil values are average regressions from 2nd and 3rd days after oiling.

The mean increment in solar utilization was 0.032 (28%) following oil coating (U of 0.115 pre- and 0.146 post-oil).

4.3.3. Computer Simulations of Heat Balance

All simulations were performed with the evaporative heat loss capacity and the heat transfer coefficient of the legs maintained at the standard setting of the heat balance model (see Appendix 1). The 'thermostat' setting was moved in such a way that the model set point (TBS) was equal to 36.5°C, i.e. the mean deep body temperature prior to oiling. This is a decrement of 0.4°C from the standard setting of the model and was considered the best representation of the data of this study. The other reference temperatures of the model were kept at the same distance from TBS as in the standard setting of the thermostat/controller.

4.3.3.1. Wind Tests

The fur conductance values used for the simulations of normal pre-oil bears were 1.8 + 0.13 V $W/m^2/°C$ where V is the wind speed. For the simulations of the post-oil animals 4 + 0.31 V $W/m^2/°C$ was used (see also section 3.2.1 re. fur measurements).

The experimental shifts from calm air to windspeed of 5 m/s took place at air temperatures near - 20°C. The model predicted decrements of about 0.1°C in deep body temperatures well in agreement with the experimental decrements of mean deep body temperatures. Also, the computer model predicted pre-oil skin temperatures in reasonable agreement with the experimental results (Figure 4-10).

Computer predictions of skin temperature for Bear 4 were comparable to experimental values, whereas predictions were slightly higher than experimental results in the remaining animals; 3°C and 2°C discrepancies for Bears 2 and 3 respectively.

For the pre-oil polar bears, the computer predictions of the increases in skin temperatures when wind exposure stopped was also in good agreement with the experimental results (Figure 4-10). However, observed increments of skin temperatures after oiling were opposite to the predicted trend.

Since measurements of metabolism were not possible during wind tests, they may not, of course, be used to evaluate the model performance regarding predictions of metabolic rate (see also section 4.3.3.2). As demonstrated in Figure 4-11, the model predicts a severe metabolic response to oiling. Thus the predicted post-oil metabolism in calm air was as high as the predicted values for normal bears at a wind speed of 5 m/s.

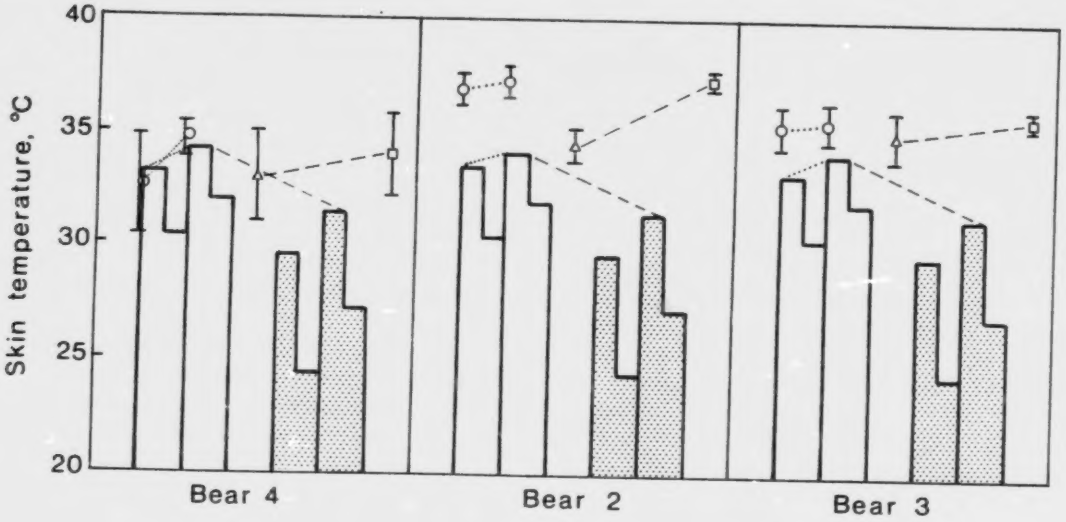


Figure 4-10. Computer model predictions of polar bear responses to wind speeds 0 and 5 m/sec at air temperature -20°C. The lefthand bar of each pair corresponds to wind speed 5 m/sec. Bars illustrating the predictions for post-oil bears are shaded. The 'steps' in the bars indicate the result of fat thickness 1 cm (low temperature value) and 0.5 cm.

Experimental values for mean skin temperature response to the shifts in wind speeds (o) are placed over the bars representing normal pre-oil bears. The mean pre- to post-oil experimental values are indicated by triangles and squares respectively. Vertical lines indicate standard deviations of the means.

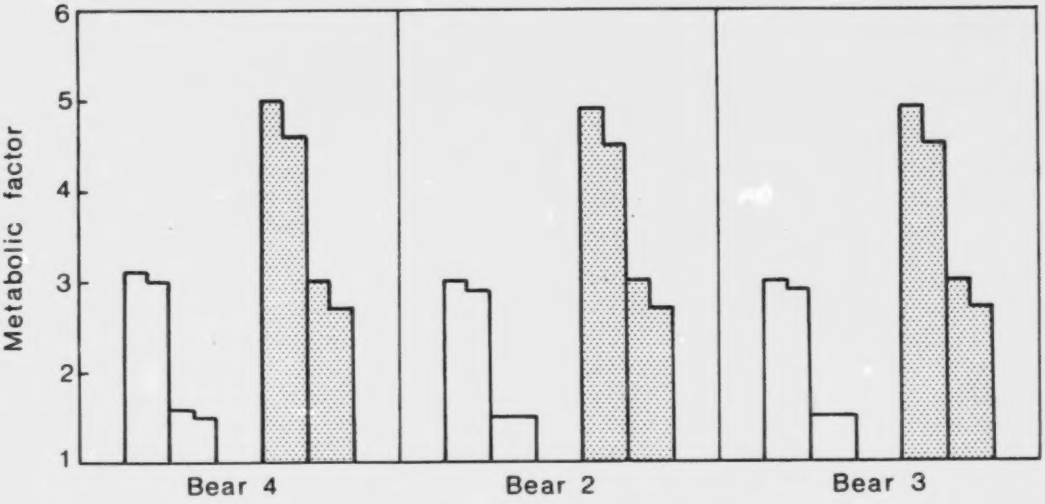


Figure 4-11. Simulations of metabolic rate expressed as a fraction of predicted basal under conditions of calm air and wind for 3 resting polar bears. Open rectangles represent normal bears, shaded rectangles represent oiled polar bears. The left rectangle of each pair is at wind speed 5 m/sec, the right side is in calm air (0 m/sec). Each rectangle assumes the animal has a fat thickness of either 0.5 cm (left-upper step) or 1 cm (right-lower step). No difference due to fat thickness was predicted in calm air for Bears 2 and 3. Ambient temperature is -20°C.

4.3.3.2. Resting Polar Bears

By using the conductance values obtained from the measurements on the fur samples, the mathematical model predicted deep body temperatures and metabolism in general agreement with the experimental values (Figure 4-12). For the present simulations the fur conductance of the appendages and the evaporative heat loss were not changed.

The skin temperatures are sensitive to the sub skin fat thickness as indicated in Figure 4-13. A fat thickness of 1 cm was chosen for the simulations of polar bear 2 and 3, while 0.5 cm was used for Bear 4. The increments in skin temperatures due to the experimental oiling of the living polar bears were, of course, not predicted by the normal setting of the computer model (Figure 4-14).

4.3.3.3. Exercising Polar Bears

Model improvements

Preliminary simulations rapidly revealed that the model with its original formula for the energy cost of walking could not produce either the rises in deep body temperature or the metabolic values recorded through the actual experiments. For example, only by doubling the walking speed in the computer was a satisfactory simulation of the 115 kg polar bear (Bear 4) achieved.

Realizing that the present data from the work tests of the pre-oil polar bears adds to the data needed to construct a general equation, a complete revision was attempted. Values were available prior to this study only for a 235 kg polar bear (Øritsland et al. 1976), and a 190 kg animal tested on the treadmill in 1979 (Hurst et al., in prep.). The results of the previous work, together with the data from Bears 2, 3, and 4, are presented in Figure 4-15. In order to produce an expression for the energy cost of locomotion for each individual polar bear studied to date, modifications and extrapolations from the available data were made. The procedure was based on two principles (Taylor et al. 1970). First, it is considered that the metabolism increases linearly with walking speed, and secondly, that the intercept value corresponding to standing still is on an average 1.7 times the basal or resting metabolic rate.

For the polar bear reported on in 1976, the statistical intercept value was well below the basal metabolic rate, while regression analysis of the data obtained in 1979 indicated a curvilinear relationship with an intercept well above two times the basal value (Figure 4-15). Consequently, linear relationships with intercepts at two times the basal values were chosen arbitrarily for the present analysis as illustrated in Figure 4-15. Thus, while respecting the available data, the statistical equations and present data points were replaced and fitted to straight lines.

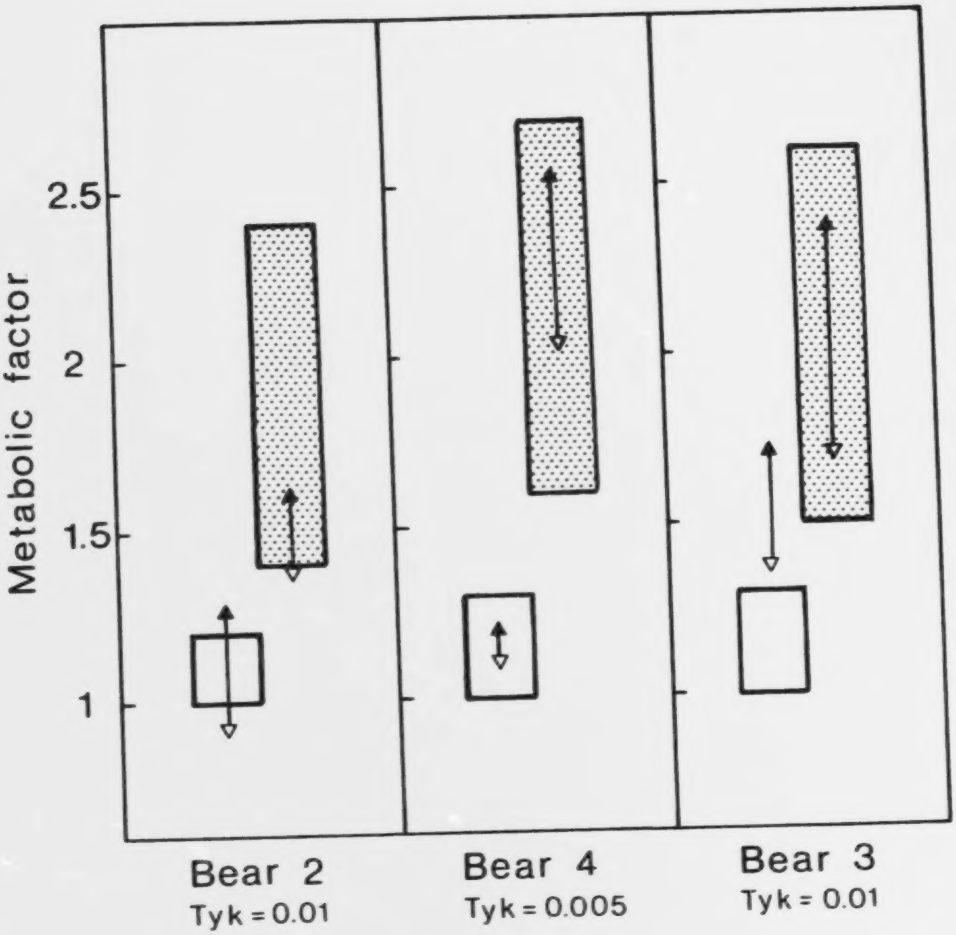


Figure 4-12 Experimental and simulated metabolism expressed as a factor of basal, for resting polar bears at air temperatures -16°C to 3°C. Experimental values are mean (closed triangle) and mean of daily minimum (open triangle) metabolic factors pre- and post-oiling. Simulated values are indicated by open rectangles for normal (pre-oil) and hatched rectangles for oil-exposed polar bears. Fat thickness was set at 1 cm for Bears 2 and 3 and 0.5 cm for Bear 4.

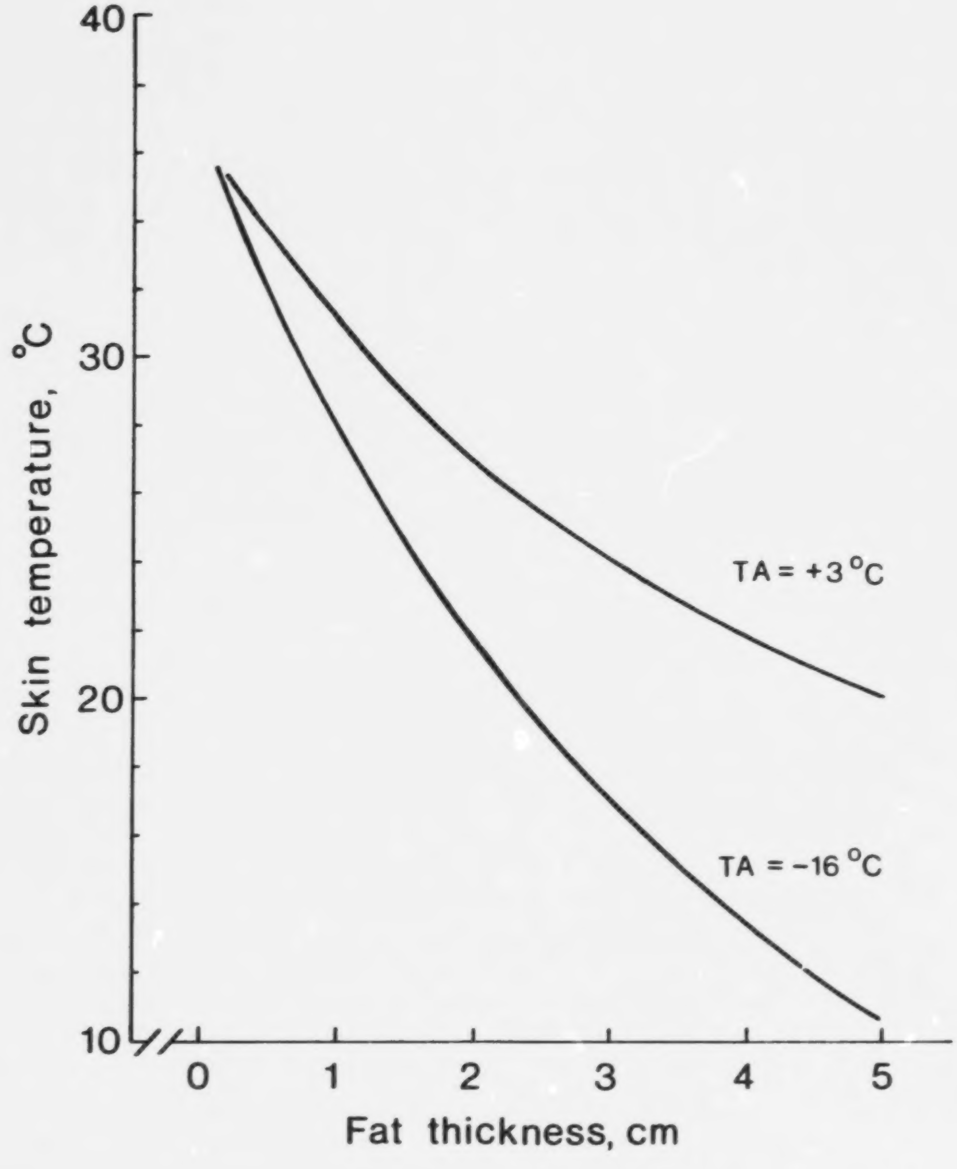


Figure 4-13. Simulations of the effect of subcutaneous fat thickness on skin temperature using polar bear 3 (body weight 163 kg) at calm air temperatures -16°C and 3°C. Fur conductance values used in the present simulations correspond to determined values for oiled fur samples (h=4).

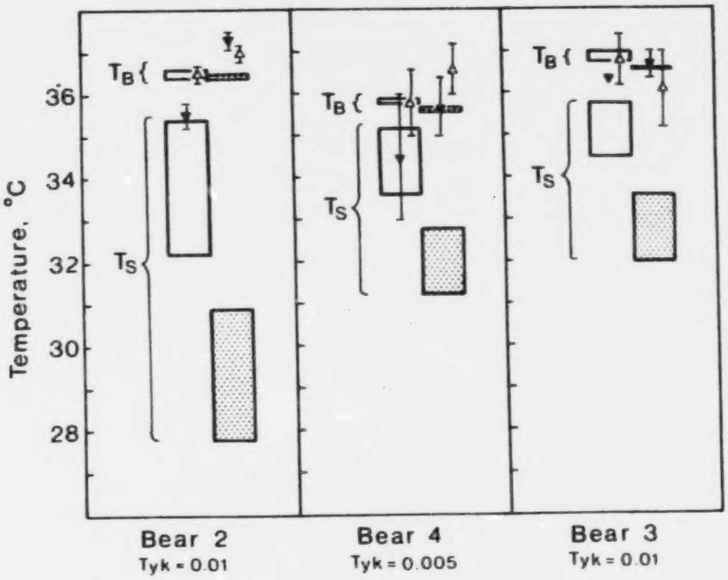


Figure 4-14. Computer simulations (▭) and experimental values of body temperatures for resting polar bears at air temperatures -16 to+3^{o}C. Experimental values are deep body temperature (Δ) and skin temperature (▲) pre- and post-oil. Vertical lines (I) represent standard deviations of the mean. Simulated ranges are indicated by (Δ) for normal (pre-oil) and (▨) for oil-exposed bears.

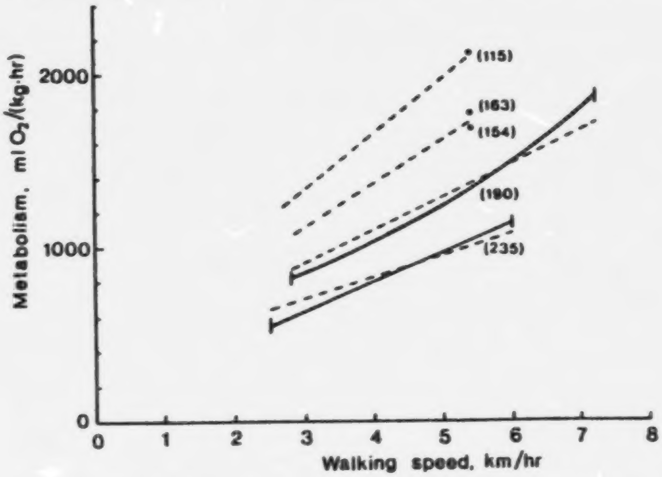


Figure 4-15. Experimental values for polar bear metabolism in relation to walking speed for the present (•) and previous (solid lines) work. Body weights are placed in brackets and extrapolations/modifications of the experimental values are presented by (- - - -). The linear extrapolations intercept the Y axis at metabolism two times the basal values.

The resulting equations were determined by reading values off the graph:

Bear	Weight kg	Equation ($ml\ O_2/kg/hr$)*
4	115	$M = 370 + 320 \cdot V$
2 and 3	159	$M = 358 + 252 \cdot V$
'1979'	190	$M = 329 + 191 \cdot V$
'1976'	235	$M = 325 + 125 \cdot V$

*where V is the walking speed in km/hr.

The slopes of the above equations are the minimum cost of walking one km, and are normally believed to be a logarithmic function of body weight (Taylor et al. 1970). In the present case a linear relationship was evident (Figure 4-16), and the line approximating the data points is described by:

$$M_m = 507 - 1.625\ W \quad ml\ O_2/kg/km$$

where W is the body weight. Obviously M_m represents the slope of the linear equation for any specified polar bear in the 110 to 240 kg range. Thus if the general equation is:

$$M = a + b \cdot V$$

where V is the walking speed, b must be replaced with M_m. The basal metabolic rate, according to Kleiber, is $70 \cdot W^{0.75}$ kcal/24 hr, which converted to ml O_2/hr is $620.6\ W^{0.75}$. For exercise, the intercept value is two times the basal and expressed per kg body weight the intercept a becomes:

$$a = 1241 \cdot W^{-0.25} \quad ml\ O_2/kg/hr.$$

Thus the total metabolism is:

$$M = 1241 \cdot W^{-0.25} + (507 - 1.625 \cdot W)V \quad ml\ O_2/kg/hr.$$

Results of simulations

Using the above equation, satisfactory computer predictions of the actual pre-oil experimental values were obtained (Figure 4-17). The mathematical model did not, however, predict the metabolic response to oil found in the experiments.

Body temperatures in the numerical model were sensitive to changes in windspeed. Assuming that the ventilation pumps and

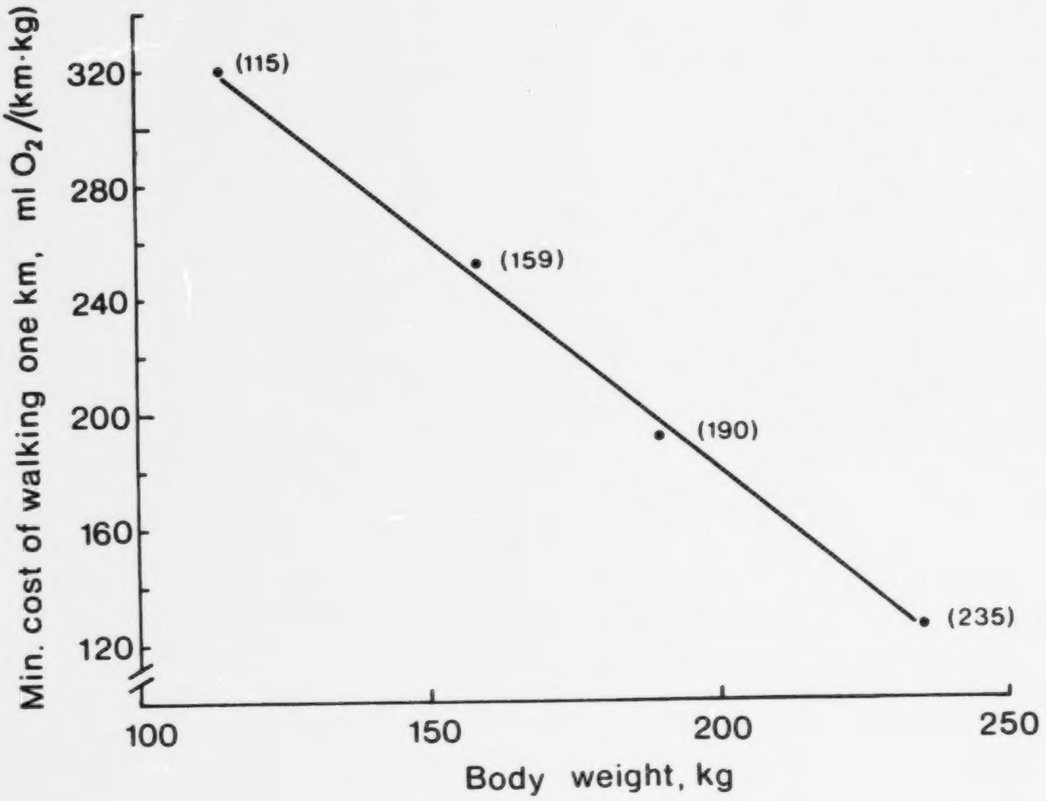


Figure 4-16. Minimum cost of walking one kilometre as a function of body size. The two higher values (115 and 159 kg) are derived from this study. The 190 kg value is derived from data of a previous study (Hurst *et al.*, in prep.) and the 235 kg value from a 1976 study (Øritsland *et al.* 1976).

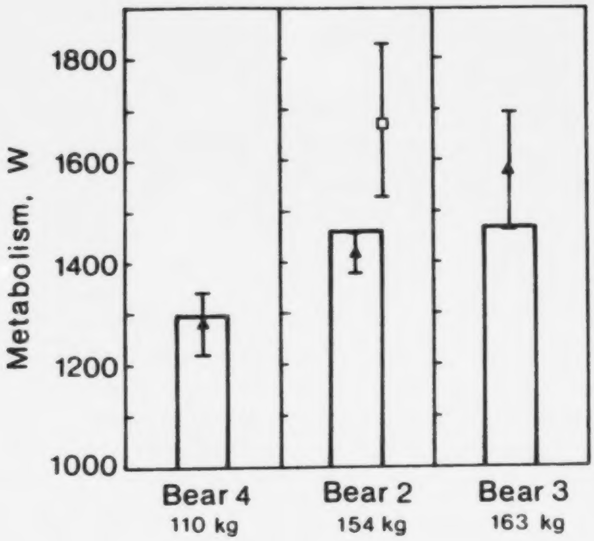


Figure 4-17. Metabolism of exercising polar bears. Histogram of computer predictions of metabolism at walking speed 5.4 km/hr for Bears 4, 2 and 3.

triangle - mean experimental values of pre-oil polar bears.

square - mean experimental value of Bear 2 after oiling.

vertical bars (I) - standard deviation of mean. The mathematical model, in contrast to experimental results for Bear 2, predicts no metabolic response to oiling.

leg movements would cause air movements, a wind speed of 0.5 m/s was selected for the simulations (Figure 4-18). The computer programme was set to display both the body temperatures and the body temperature increments after 30 minutes walking at 5.4 km/hr. The result of a single simulation is presented in Figure 4-19, and an integration of the simulations and experimental results is presented in Figure 4-18. It appears that the computer predictions of the pre-oil polar bears are adequate while the metabolic and temperature responses to oiling of Bear 2 were not in agreement with the simulations.

4.4 Discussion

4.4.1 Living Animals

4.4.1.1 Oil Contact

The live animal experiment simulated natural conditions in that the polar bears entered a saltwater pool covered with a slick of aged crude oil. It was not designed, however, to explore the behaviour of the animals under conditions of free choice. This would require an experimental design such that free access to oil-contaminated water was allowed following a period wherein the bears had become familiar with the structure and water. It might make use of an incentive i.e. seal blubber accessible only through swimming, or of a pool subdivided into oiled and non-oiled surfaces (Williams 1978).

Oil adhered to the fur on contact, suggesting that physical fouling of fur would occur relatively rapidly. Oil distribution was uneven and depended on the point of contact with the water oil surface; however, with time the oil did become more evenly worked into the fur surface. The results suggest that a polar bear crossing an oil fouled lead would become physically contaminated by a substantial amount of oil.

4.4.1.2. Wind Tests/Behaviour

Behaviour

The behavioural response to wind of reducing activity and assuming an energetically more efficient posture is in agreement with predicted behavioural thermoregulation (Øritsland 1970). The probable overlap, however, of thermoregulatory and toxicity-induced behavioural responses makes a quantitative interpretation of oil dependent activity levels unrealistic.

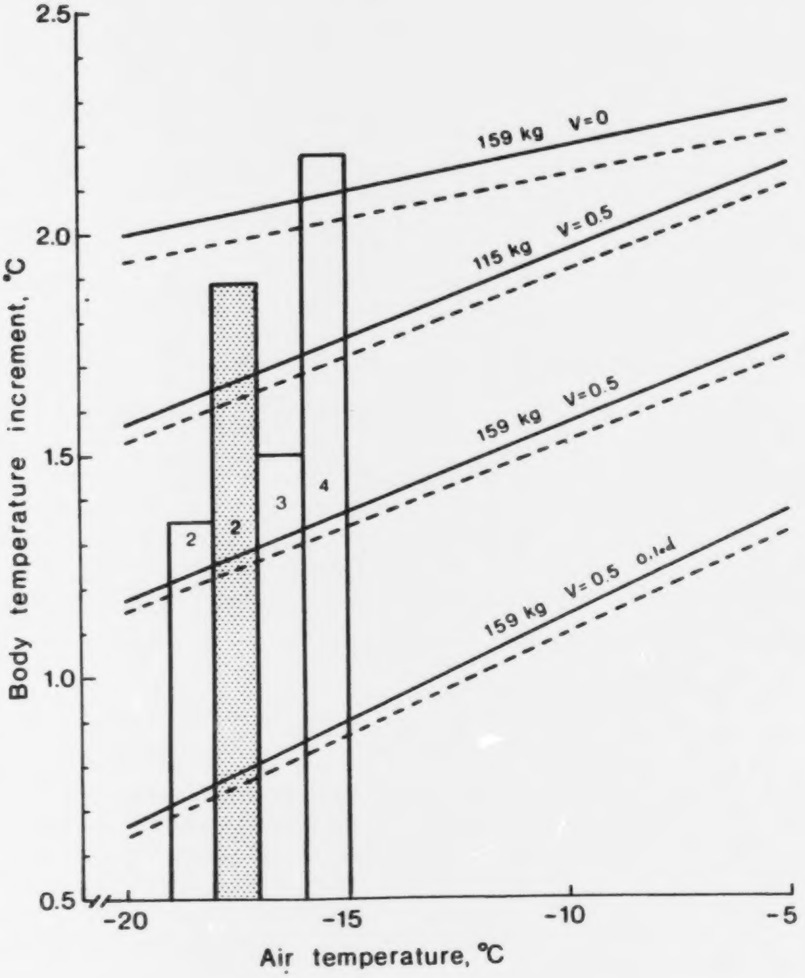


Figure 4-18. Computer predictions of body temperature increments resulting from 30 minutes of walking at 5.4 km/hr. The solid and broken lines indicate deep body and skin temperature increments respectively. Body weights (kg) and wind speeds (m/sec) are placed at the lines. The vertical bars indicate the experimental results from polar bears 2, 3 and 4 including the post-oil test of Bear 2 (shaded). A wind speed of 0.5 m/sec is considered realistic for the experiments.

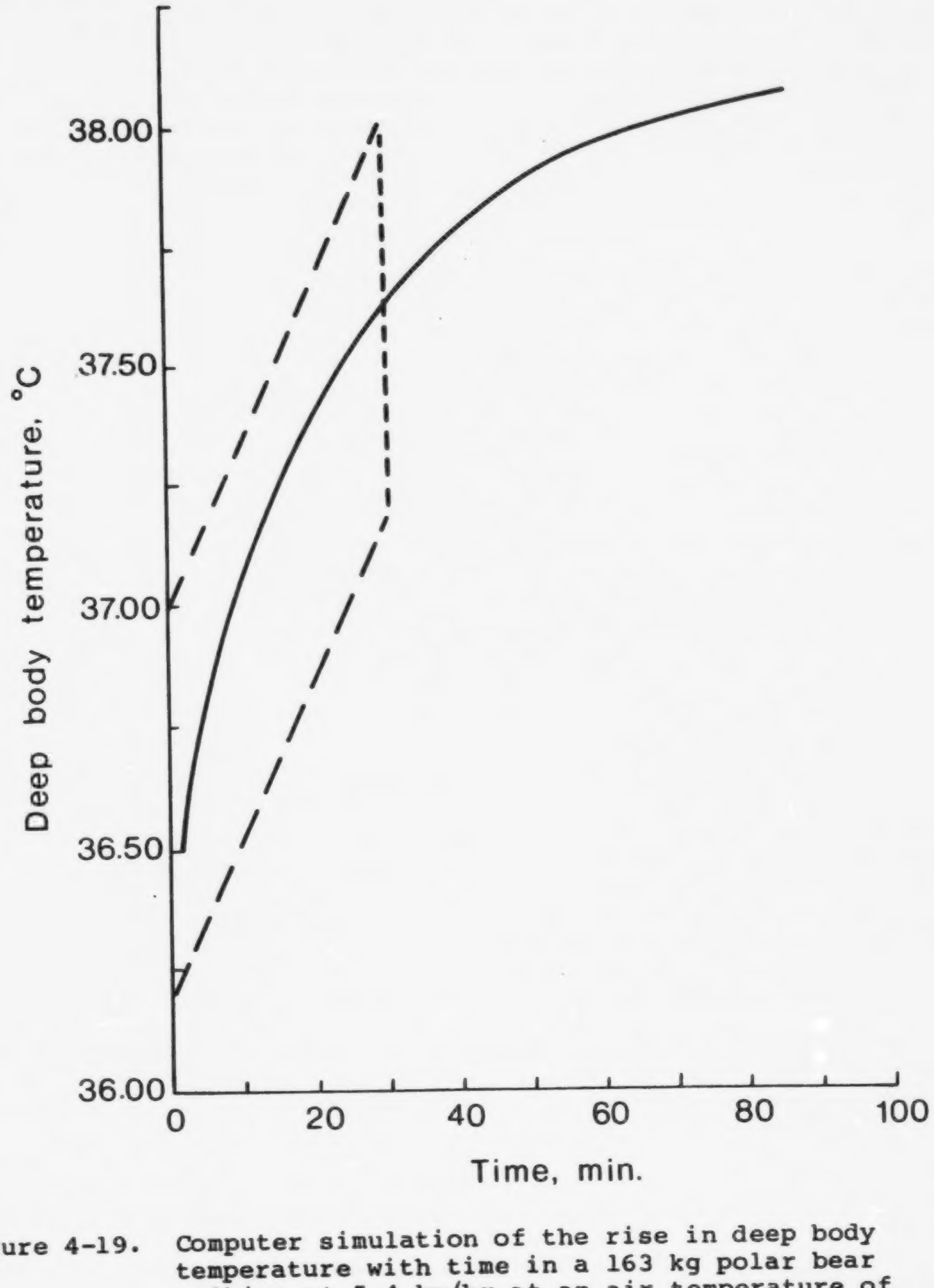


Figure 4-19. Computer simulation of the rise in deep body temperature with time in a 163 kg polar bear walking at 5.4 km/hr at an air temperature of -20°C. The broken lines encompass the range of experimental results obtained from 30 minute exercise tests of three polar bears (pre-oil).

Perhaps the most dramatic behavioural manifestation was the duration and intensity with which the polar bears ingested crude oil. Grooming began immediately upon emergence from the oiled pool and continued with little relief for several hours (See Figure 4-4). The internal toxic effects are outside the scope of this chapter of the polar bear oil study. However, serious consideration must be given to the critical nature of the first 12 hours or less following oil contact; particularly when considering the development of contingency plans in the event of an arctic oil blowout or spill.

The polar bears in this study (with some exception in Bear 4) made little use of the snow or the cage structure to physically remove oil from the fur, a behaviour which may or may not be indicative of the response by free ranging polar bears in natural snow conditions.

Also of interest was a behaviour exhibited by Bears 3 and 4: that of apparently seeking out and consuming oil remnants prior to exposure in the pool. This would seem to confirm scattered and unpublished reports of polar bears in the wild scavenging snow machine oil. However, this is a subject for qualification and investigation under controlled conditions before any extrapolation to free ranging polar bears may be made with any degree of confidence.

Temperatures

The apparent lack of periodicity in body temperatures corresponding to the wind periods (Figures 4-5 and 4-6) has not yet been subject to detailed scrutiny employing data reduction or moving averages techniques. The slight, but statistically insignificant depression of deep body temperatures during wind exposure is interesting. Such a decrement in deep body temperature conforms with the predictions of the computer model. Body temperature variations may be very slight and still reflect considerable changes. Thus, more accurate measurements are now needed.

Ths use of sensitive micro-electronics, for example activity load cells in combination with an on-line computer, may allow resolution of hundreds in lieu of tenths of a degree in future experiments.

The depression in deep body temperatures after emergence from the pool (Figure 4-5) is consistent with previous results from swimming and walking polar bear cubs (Øritsland 1969). Such decrements in deep body temperature after swimming or immersion in water are due to the cooling of peripheral body tissues while the animal is in the water.

The cooling reflects heat loss to the water and is amplified by the normal need for a reduction in peripheral blood circulation under such circumstances. The result is that the body tissues are mobilized as an insulative defence against heat loss from the body core. Upon emergence from the pool, the muscle and fat insulation is needed less, and blood is circulated through the tissues and returns cooled to the body core. Consequently, a drop in deep body temperatures takes place after the period in the water. Thus, the observed decrements in deep body temperature just after oiling are not necessarily explained as an effect of oil, but rather as a normal response to water immersion.

The same physiological mechanism may also contribute to the wider variance in body temperatures after oiling.

Analogous to water immersion, the reduction in fur insulation due to oiling will enhance the cooling effect by wind. The wind periods should thus trigger emphasis on sub-skin insulation with a corresponding drop in skin temperature as appeared for Bear 4 (Figure 4-6). On the other hand, the observed change in activity pattern may contribute to oscillations by increasing body temperatures. Hence the increased variation in body temperatures after oil exposure has two components of normal temperature responses magnified by oil fouling: bursts of activity will cause increases, and intermittent wind exposure will cause periodic decreases. Since the two components have different time constants and are counteractive, the resultant body temperatures cannot be expected to consistently reflect moderate periodic changes in ambient cold. Also the general elevation in skin temperatures, indicative of a reaction to oil, may dampen normal thermoregulatory responses.

4.4.1.3. Resting Polar Bears

The metabolic values obtained from the pre-oil polar bears in the respiration chamber were in general agreement with what may be expected from Kleiber's formula of basal metabolism i.e. the pre-oiling bears may be considered normal. Likewise, the body temperatures were within ranges reported earlier. (See heat balance model Appendix 1.)

As mentioned above, the shifts in metabolism and temperatures due to oil exposure must be considered in the context of several principles:

Obviously the twofold reduction in fur insulation, as determined for the fur samples (section 4.3.2) would cause a compensatory increment of the heat production if the oil-fouled animals were otherwise normal. Such normal animals also would compensate by reducing their peripheral blood circulation, causing a corresponding increase of tissue insulation and reduction of skin temperatures. The present oil-fouled polar bears, however, exhibited increased skin temperatures. Therefore it may be surmised that either the oil caused a malfunction of the thermoregulatory system or all three animals were emaciated at the start of the experiments. Observations of body weight in relation to size and estimates of skin fold thickness indicated a normal body composition for all bears. Clinical parameters confirm that animals were in a healthy condition prior to oil exposure (see Chapter 6). Hence a malfunction of the thermoregulatory system is indicated.

The changes in deep body temperatures were not consistent and were large enough to perhaps suggest a pyrogenic, i.e. fever-causing, effect of oil.

In the present context the metabolic elevation caused by the oil may be considered due to 1) a direct effect on the minimum level of energy turnover of the tissues, 2) a normal metabolic compensation for a reduction in fur insulation, 3) a skin reaction in combination with the effects of reduced insulation or, finally, 4) a combination of all three mechanisms.

The increments in skin temperatures suggest that there was a skin or peripheral tissue reaction to the oil. Whether this reaction was caused by ingested oil or was a direct response to oil-coating cannot be evaluated on the basis of the present data.

4.4.1.4 Exercising Polar Bears

Metabolism and temperatures

The metabolic values obtained for the pre-oil exercising polar bears are higher than what may be expected from general expressions scaling the energy cost of locomotion (Taylor et al. 1970, Fedak and Seeherman 1979): Considered alone, such high metabolic values at a walking speed 5.4 km/hr would seem doubtful. As discussed for the computer simulations of the exercising bears, however, the circumstantial evidence provided by the temperature measurements corroborates the present results.

After oiling, the exercise metabolism of the only polar bear tested (No. 2) reached an even higher level. For the resting animals, an increased metabolism after oiling may be considered a normal thermoregulatory response. At the present levels of exercise, however, the heat production irrespective of oil is nearly 10 times the basal levels. Also exercise per se does not significantly alter the fur insulation. Thus from a thermoregulatory point of view the work load generates excess heat - and a metabolic response to oil contamination of fur is not at all expected.

The pre-oil elevation of deep body temperature during exercise is in general agreement with previous observations (Best 1976, Hurst et al., in preparation). For humans, the equilibrium deep body temperature increases linearly with work load (Astrand and Rodahl 1970) while for polar bears there is a curvilinear relationship. The greater rise in deep body temperature during post-oil exercise than during pre-oil exercise is not in agreement with a normal thermoregulatory response, but conforms with findings for humans with fever (Grimby 1962): " - - during the rising phase of fever there was a greater rise in body temperature during exercise than during either the afebrile state or the steady febrile state." (See Bligh 1973 p. 235). For normal Homo sapiens, the linear equilibrium deep body temperature versus work load relationship appears independent of considerable variations in ambient heat load, while for polar bears a positive correlation with ambient temperature is indicated (see mathematical model). Thus, for the exercising polar bear tested, the rise in body temperature after oiling suggests a pyrogenic effect of oil. In addition, the smaller post-oil skin temperature increment due to exercise indicates a skin reaction with increased blood circulation not subject to normal thermoregulatory control.

The post-oil exercise increment of metabolism is not in agreement with an ordinary fever response. (Grimby 1962: "During fever, the exercise metabolism of man is expected to be normally dependent on work load, while the extra rise in deep body temperature is interpreted as reflecting a change in the action of the thermal controller and/or a reduced capacity for heat dissipation from the skin." Therefore, the polar bears' metabolic response to oil as tested by controlled work loads seems abnormal both from a thermoregulatory point of view and in the context of a fever response.

4.4.2. Fur Samples

The pre-oil insulative values were very comparable for pelts from each of three animals. This was not unexpected since the pelts were collected from three polar bears of comparable age and size and at the same time of year. Calm air conduction of fur 1B, a more posterior dorsal section, was somewhat lower than that of the remaining three samples. The difference may reflect a topographical difference over the body surface, a distribution recognized, but not previously quantified.

The calm air conduction values in this study (h = 1.75 $W/m^2/°C$) lie between the range previously reported for polar bear summer fur (Øritsland 1975) (h = 3.6 $W/m^2/°C$) and winter fur (Scholander et al. 1950) (h = 1.0 - 1.4 $W/m^2/°C$). Hart (1956) demonstrated a 32% seasonal change in polar bear fur insulation.

The effect of oil on insulation was dramatic, with substantial conductance increment factors of 2 - 4 recorded for all oiled furs. The dependence of conduction on wind was reasonably well fit by a straight line relationship (average R^2 = 76.2). Oil coating caused not only an increase in calm air conductance (h), but also a significant increment in the wind coefficient (b = 0.11 pre-oil vs. 0.56 post-oil).

Wind chill (Øritsland 1974) would therefore be more severe for an oil-fouled polar bear.

Under normal conditions conduction values, since they are expressed as a function of temperature gradient, are relatively independent of ambient temperature. In the course of the present study it became evident that oil introduces new considerations, primarily because oil viscosity is temperature dependent (Figure 4-3). Thus, for the oil-exposed fur, ambient temperature and solar radiation, in combination with sub-skin temperature, determine the physical characteristics of the oil coating as well as the heat load at the skin surface.

Since the physical matting of the fur appears directly related to viscosity, consideration must be given to not only the amount and physical nature of the oil but the temperature of the oil-fur surface. There is also a temporal consideration, since at present high experimental temperatures at least, a fraction of oil would 'melt' off the fur with time. Of course, in a live encounter, oil would also be partly removed by behavioural means (see section 4.3.1.). The more viscous oil (Base 2500) did not 'melt' as noticeably, and as a result, physical matting and conductance values remained relatively consistent over time (Figure 4-8).

Such a comparably viscous oil may best approximate a potential natural encounter since most oils are viscous at the water and air temperatures to which a polar bear is normally exposed. Such physical considerations could be better controlled and quantified by performing fur-oil conductance measurements under low environmental temperatures.

The solar utilization coefficients determined for pre-oil pelts are in good agreement with those obtained for polar bears (Øritsland 1975). Although no wind-dependent solar utilization relationship was apparent in this study, such a relationship may be obtained under more rigorous experimental conditions.

The observed increment in solar utilization following oil exposure is in general agreement with results of a similar study using ringed seal pelts (Øritsland and Smith 1975). Polar bear hairs have a complex medullary structure (Øritsland and Ronald 1978) and transmittance and reflectance measurements at the single hair level are necessary before the mechanisms of the observed shift in solar utilization can be elucidated.

The results of this study suggest that under conditions of relatively constant air temperature and wind speed, an abrupt increase in solar radiation could have a profound effect on the insulative value of the fur. Solar radiation at low environmental temperatures may save energy by lowering the critical temperature and at high temperatures put an extra burden on heat dissipation (Schmidt-Nielsen 1964). The change in viscosity coupled with the high solar utilization, suggested by the results of this work, would act as a barrier against heat loss under sunny conditions. Consequently the functional insulative value of an oil-fouled fur may exhibit more dramatic shifts with changing sunshine, wind speed and air temperature than that of normal polar bear fur.

As noted above, the magnitude of such shifts however, may only be determined by conducting fur measurements under a wider range of air temperature than the present +16 to +35°C.

Polar bear fur, in contrast to some other aquatic mammal fur, allows water to penetrate to the skin surface, dislodging all air. Water is normally shed easily upon shaking, however, so that insulation is readily restored (Scholander *et al*. 1950). Oil, of course, is not so easily removed. Polar bear fur is of relatively poor insulative value for its thickness yet is, for example, comparable in overall insulation to that of a red fox or husky dog. It is of extreme importance to the individual animal, and thus a change in insulative properties of the magnitude found in this study represents a serious stress to the animal's heat balance and thermoregulatory stability.

4.4.3. Computer Simulations

4.4.3.1. Wind Tests.

Skin temperature is determined by the metabolism of the animal and is also dependent on both fur and fat insulation. Therefore, on the basis of the wind tests alone, it is not warranted to attempt alleviating the discrepancies between model predictions and experimental results by adjusting the model to conform with each individual polar bear. The simulations of the wind tests were performed with the model after exploratory simulations of all aspects of all project experiments had been performed. Thus, having a model considered to be a fair approximation of real-world polar bears, the agreement between model predictions and experimental data from the pre-oil animals substantiates the fidelity of the model.

Next, accepting the model as a good representation of living polar bears, the observed rises in skin temperatures after oiling must be considered as clearly abnormal skin reactions. The thermal consequences of such skin reactions must, in principle, be to further increase the metabolic response to oil. The present simulations suggest that the effect of oil on the fur insulation alone will induce an elevated energy drain in a resting polar bear comparable to the energy cost of slow walking (2 - 3 km/hr). The skin reaction will act to further increase the energy drain. A quantitative evaluation of such an effect has not been attempted in the present work. Such analysis seems possible, however, and would require that a limiting function was forced onto the skin temperatures in the computer programme.

4.4.3.2. Resting Polar Bears

Although Bear 3 had somewhat higher metabolism and skin temperatures than predicted by the model, the overall agreement between pre-oil experiments and computer predictions is satisfactory. The higher than predicted pre-oil skin temperatures of Bear 3 could be alleviated by increasing the model fat thickness by only 0.5 cm. Such a fitting of the model to the individual bear is considered unwarranted, however, because the higher experimental values may well be explained by restlessness of Bear 3 in the respiration chamber during the pre-oil period.

Thus, according to the computer simulations, the observed change in metabolic and body temperature values can be largely explained as a thermoregulatory response to the reduction in fur insulation caused by oil. The model does not predict, of course, the observed increment of mean skin temperature following oil exposure. The increment may be considered a local and/or centrally controlled abnormal skin reaction. It seems that the model may also be used to evaluate the nature of such thermoregulatory malfunctions when experimental data on evaporative heat loss becomes available to further refine the model.

The present simulations predict two possible responses dependent on the gross energy stores of the individual animal. A polar bear with thick layers of sub-skin fat may suffer severe skin freezing if its fur becomes fouled with oil. On the other hand, a lean bear in the same situation may respond with a strong elevation of energy turnover, thereby moving further into a state of detrimental energy balance.

Lower critical air temperatures are conventionally used to describe adaptations to cold (See heat balance model Appendix 1). Model predictions of the relationship between the critical air temperature and fur conductance are presented in Figure 4-20, emphasizing the detrimental effects of oil on polar bears' ability to rest under cold conditions.

4.4.3.3. Exercising Polar Bears

Obviously all factors included in a mathematical model simulating real world observations must conform with experimental results. It was the biological limitations on other factors such as total heat capacity, surface area constants, evaporative heat loss capacity, etc. that precipitated the reanalysis of the metabolic cost of walking. It appears from Figure 4-16 that the straight line relationship between body weight and slopes of weight-specific equations for walking is justified from a statistical/curve fitting point of view. It should be realized, however, that such a linear change of slope becomes biologically impossible if applied to large polar bears: A body weight of 312 kg theoretically produces zero slope, implying the entertaining thought that polar bears of that size will not increase their metabolism with walking!

On the other hand, for polar bears weighing about 100 kg, the present equation for energy cost of walking predicts considerably higher values than the equations of Taylor et al. (1970) while it is in reasonable agreement with the equation of Fedak and Seeherman (1979) for body weights approaching 200 kg.

Thus, the present equation should be considered applicable to polar bears with body weights between 100 and 250 kg only. The same equation invites the speculation that polar bears are adapted to maximum efficiency, i.e. low energy consumption as adults, while the energy requirements for the two first years of life are high in comparison with other large mammals.

After revision, the computer model simulations of metabolism at walking speed 5.4 km/hr were in good agreement with the experimental results from the pre-oil polar bears (Figure 4-17). However, the model did not predict the post-oil increments exhibited by Bear 2.

The rise in body temperatures during 30 minutes of exercise were, according to the model, sensitive to wind speeds (Figure 4-18). Accepting that a wind speed of 0.5 m/s is reasonable for simulations of the conditions in the treadmill chamber, good agreement between predicted and real world temperatures was obtained for the pre-oil polar bears (Figure 4-19).

The simulations of the post-oil response in deep body temperatures of Bear 2, however, are opposite to the experimental results. Experimentally, deep body temperature increments were recorded while the model predicted

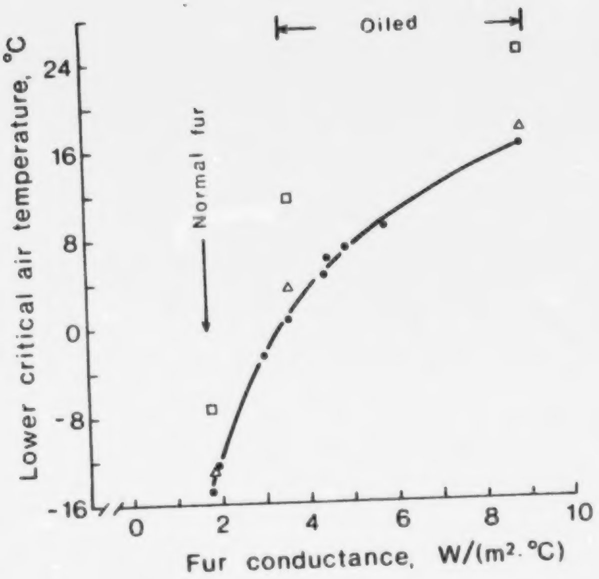


Figure 4-20. Simulated lower critical temperatures for polar bears of 2 body weights over a range of fur conductance values. Lower critical temperatures are presented for a 160 kg bear with fat thickness of 3 cm (circle) and a 110 kg polar bear with a fat thickness of 3 cm (triangle) and 1 cm (square). Normal and oiled conductance values are derived from fur sample studies (section 4.3.2). Conditions are that the animals are at rest and that wind speed and solar radiation are equal to 0.

depressions. Bearing in mind that the computer simulations of the normal bears are in agreement with the experimental results, the discrepancy after oiling emphasizes that Bear 2 developed a thermoregulatory malfunction. Whether this malfunction concerned the control of skin blood flow only, or the control of both metabolism and skin circulation, has not been subject to computer analysis within the frame of the present study.

Since the thermoregulatory malfunction includes both reduced fur insulation and a skin reaction (hyperaemia), it must result in a very high heat loss from free roaming oil-fouled polar bears. Such a heat loss may prevent skin freezing injuries, but may also, because it cannot be switched off, cause polar bear death due to exposure.

4.4.4. General Discussion

Both the metabolic and temperature values of the resting pre-oil polar bears suggest that the experimental animals were in normal health prior to oil exposure. The determined pre-oil metabolism of walking polar bears is interesting and supports in general Fedak and Seeherman's (1979) reconsiderations of the general equations for the energy cost of walking (Taylor et al. 1970). Metabolism in relation to controlled work loads has been examined for only a few large homeotherms. Therefore a general evaluation of the "normality" of walking polar bears would suffer from lack of data base and the discussion would have to focus on the basic concepts of activity metabolism. Such a discussion is outside the scope of the present work. It should be noted, however, that the energy cost of locomotion in polar bears may be expressed in terms of food energy requirements. Thus further analysis of activity metabolism of polar bears also concerns the energy balance between polar bears and seals in general.

The post-oil experiments revealed a consistent pattern of increased metabolism and body temperatures resulting from oiling. The use of relatively large transmitters for measurements of skin temperatures involves some practical difficulties in interpretation regarding the transmitters' effect on local blood circulation. Also, the extent to which the recorded temperatures represent the skin surface, and not more central positions on the temperature gradient, should be investigated. Now, after the significant skin temperature response to oiling indicated by the present experiments, a study of blood circulation and temperature gradients in the vicinity of chronically implanted skin temperature transmitters should be undertaken.

It should be noted that the principal thermoregulatory responses to oil elucidated by the present work are also relevant to seals and other large mammals. Particularly for arctic homeotherms, maintenance of normal deep body temperatures in oil-fouled animals may occur concurrently with severe thermal stress. Such maintenance of deep body temperature may be accomplished by mobilizing the peripheral body tissues to take part in the total insulation against heat loss.

Although such compensation for loss of fur insulation may successfully maintain stable deep body temperatures, such stability must be paid for by a reduction in skin temperature that may easily result in freezing injuries. The alternative response to oil indicated by the present work, i.e. an obligatory rise of skin blood circulation, is paid for by an increase in metabolism which serves to accelerate the exhaustion of body energy stores. In either case, significant changes in animal behaviour must be expected. The direct effect on polar bear hunting behaviour or seasonal movement patterns should be seriously considered. Also, oil-induced changes in the haul-out behaviour of seals, for example, may affect the exploitation of this group of animals both by polar bears and arctic man.

The notion that oil will cause fever in polar bears is supported mainly by the treadmill tests of Bear 2. This emphasis on the work load tests is appropriate since the responses of the resting animals may be assigned an alternative explanation: It is possible that the agreement between the observed and computer predicted metabolic values of resting polar bears were coincidental. Oil detoxifying mechanisms and non-specific stress responses could have elevated the metabolism to levels that also happened to be sufficient for thermal balance. Hence a compulsory rise of the basal metabolic rate due to oil ingestion is conceivable. If this is true, the metabolism at a given walking speed would be elevated, resulting in a higher than normal body temperature. Such a temperature increment, due to a rise in metabolism, would not imply the same health hazard as a temperature rise due to fever. Fever represents a resetting of the "thermostat" and the resulting controlled high body temperature may, if the condition goes on too long, result in brain damage. A general metabolic elevation due to detoxifying metabolic activities in the body tissues, however, may more easily be sensed by the animal and alleviated by reducing the work load. Also, a general metabolic elevation will at all times be counteracted by normal increments of peripheral blood circulation and evaporative heat loss. The possibility of a separate skin reaction further complicates the situation. In order to avoid harmful procedures in handling of polar bears exposed to oil, the above considerations should be pursued by further computer analysis and experimental work.

In its present form the computer model may be used to demonstrate the thermoregulatory effects of oil irrespective of eventual skin or fever reactions. For example, a normal 160 kg polar bear tolerating a doubling of the basal metabolism while resting will be severely affected by oil (Figure 4-21). In calm air conditions, its air temperature tolerance is shifted from about -55°C to about -25°C while at wind speed 5 m/s the upwards shift is more moderate (-15 to -7°C).

In the present situation, where practically no information exists on polar bear activity patterns in relation to weather conditions, the model should be used to determine the integrated effect of solar radiation, wind speed and air temperature on polar bear metabolism. Such weather indices are the basis for understanding the thermal aspects of polar bear behaviour.

4.5 Conclusions

Measurements on living polar bears elucidating metabolic (energy consumption) and temperature responses to changing windchill and work load have been performed. The controlled nature of the experiments, undertaken both before and after exposure to crude oil, facilitates comparisons. A consistent pattern emerges, with significant rises in both metabolism and skin temperature resulting from exposure to oil. The rises in metabolism are considered as mainly compensatory responses to cold stress.

The results of experiments on living polar bears were complemented by studies of fur samples. Oil coating resulted in a reduction of fur insulation to half or less than half of the normal value. The oil also caused a more rapid deterioration of the insulative value with increasing wind speeds. Solar heating of the skin increased by 28% as a consequence of oiling. The physical behaviour of the oil caused the polar bear fur to lose its normal independence of absolute air temperature.

Collectively, the results suggest that after oil exposure a free ranging polar bear's thermoregulatory balance would be stressed, and more radically susceptible to changes in environmental temperature, wind speed and sunshine.

The results from the experiments on both fur samples and living animals were integrated in a computer model of the heat balance of polar bears. The experimental results from the pre-oil bears confirmed the validity of the model and strengthened the potential serviceability of the model as an ecological tool.

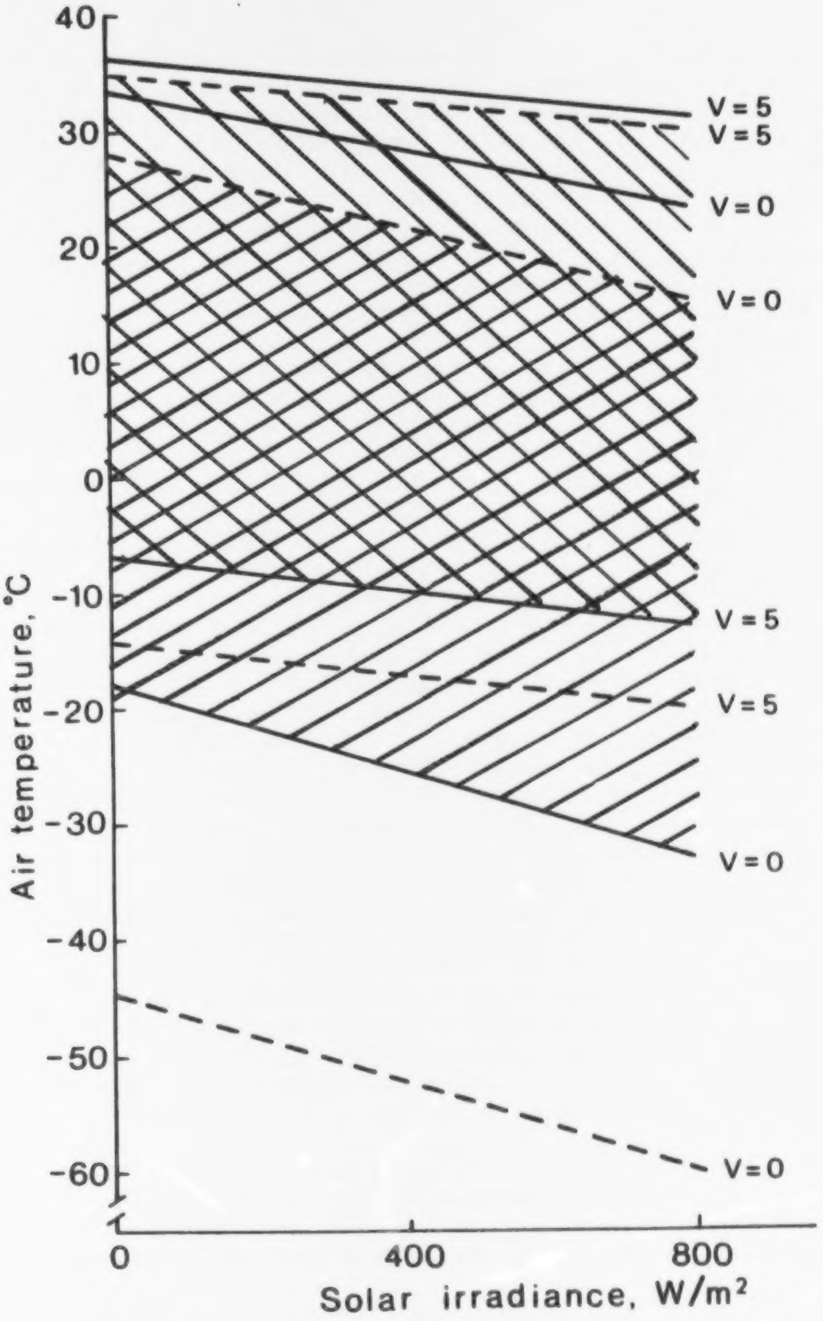


Figure 4-21. Computer model prediction of the weather tolerance of a 160 kg polar bear before (broken lines) and after (solid lines) oil exposure. V is wind speed in m/sec, fat thickness is 3 cm and the tolerance criterias used are 1) stable deep body temperature and 2) metabolism less than two times the basal value.

Both conventional physiological considerations and computer analysis suggest that oil-fouling will cause a severe cold stress in polar bears. Apart from the compensatory energy drain mentioned above, cold stress may alternately cause a physiological response that will result in freezing injuries. It also appears that the Midale crude oil used in these experiments may have had an effect similar to fever causing agents (pyrogens). In addition, there was a considerable skin reaction due to oiling.

Since the study was not designed to isolate external from internal oil exposure, it could not be resolved whether the skin and fever reactions were caused by ingested oil or by oil uptake through the skin.

The relatively sparse data base with respect to pyrogenic effects of oil makes conclusive statements concerning fever unadvisable. The possible manifestation of such a response has, however, serious implications and is discussed. The apparent fever-mimicking role of oil necessitates unusual care in handling oil-contaminated polar bears - as otherwise acceptable techniques of bear capture and transfer employing helicopters may cause dangerous overheating in oil-stressed animals. It must be assumed that the oil-induced changes in heat balance will cause changes in polar bear behaviour.

Similarly, the possibility of physiological and behavioural responses to oil by oil-exposed seals should be considered; it must also be assumed that arctic marine oil pollution will affect the utilization of seals by polar bears and by arctic man.

The present study has provided both experimental data and an analytical tool useful in assessing the potential severity of arctic marine oil pollution. It may also aid in designing contingency plans useful in the event of an oil spill or oil well blowout in polar bear living areas.

4.6. Acknowledgements

The range of agencies and personalities involved in this international project is considerable. Therefore, we would first like to offer a general thanks to all those in the area of animal care, and those of administrative agencies: Provincial, Federal and International, for their level of understanding of the seriousness of this arctic problem. We are particularly indebted to representatives of the Canadian Government and the Government of N.W.T., especially Ray Schweinsburg who supported the study since its inception.

Winnipeg and Churchill representatives of the Manitoba Dept. of Renewable Resources contributed valuable assistance whenever possible. A personal thank you is extended to the local citizens of Churchill for their continued help despite the sensitive nature of the study.

Finally, we would like to acknowledge those dedicated people who made the concept a working reality; the members of the Polar Bear Project, Churchill, including Dave Belme, Steve Hansen, Marty Leonard, Paul Ratson and Les Stegenga. Also, sincere thanks to those in Oslo: Trond Backstrom, Chris Cuyler, Lars Folkow, Anne Margrete Holst and Lill-Ann Øritsland who assisted the project through its final stage.

5. OIL CHARACTERISTICS, ACCUMULATION AND CLEARANCE

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5.1. Introduction

5.1.1. General Considerations

Adequate characterization of contaminant oil is predicated on a number of requirements. One is that the composition of a petroleum, that is, the relative proportion of hydrocarbon fractions, needs to be known in order to determine in a comparative and predictive sense the toxicity of the oil. Different hydrocarbon fractions do differ in their toxic effects; the smaller molecular weight fractions are associated more with an acute effect, often neurological or hepatotoxic in nature. The large molecular weight fractions tend to be associated with chronic or latent effects, including carcinogenesis. Another consideration is that the different fractions show different solubility and retention characteristics in the environment and in the organism. This will then relate directly to the toxic potential of a spilled oil.

All crude oils contain three general classes of hydrocarbons - alkane, cycloalkane and aromatic (NAS, 1975 - Workshop). The toxicity of crude oils appears to correlate with the relative content of kerosene, naphtha and gasoline (Buck et al. 1976). The most toxic components of crude oils and crude oil distillates are in the aromatic hydrocarbons group which, among others, include benzene and its various alkyl derivatives such as toluene and xylene in the low boiling fraction, and naphthalene in the higher boiling fraction (Gerarde 1963, Chap. 30). The low boiling alkanes and cycloalkanes are of comparable toxicity and are generally considered to be less toxic than the aromatics (Gerarde 1963, Chap. 28, 29).

The most detailed characterization of petroleum into its component hydrocarbons is generally carried out by gas chromatographic methods, best coupled with mass spectroscopy. This allows for a fingerprinting of the contaminant oil, particularly important if the source of the contamination is not known. Such methods, as descriptive as they are, are not, however, either fast or economical under circumstances when a large number of samples need to be processed; other methods, for instance fluorometry or spectroscopy, may be implemented.

As a general experimental principle, it is necessary to establish the fact of uptake in the organism. By a distribution assessment, it may be possible to identify particularly sensitive or target tissues in a true dose-cause-effect relationship. By knowing change in levels of the contaminant hydrocarbons over time in various body tissues, fluids, and products, clearance may be related to recovery parameters.

In this study it was necessary to characterize the contaminant crude oil by its component hydrocarbons to be able to compare its toxicity effects with other crude oils. Physical characteristics were also considered, as were aspects of "weathering", that is, exposure to air and water. A more efficient analytical technique was needed to follow the accumulation and release of petroleum from the experimentally exposed polar bears; fluorometry was felt to be an effective analytical tool.

5.1.2. Physical and Chemical Properties

Crude oil, such as the Midale crude of this study, is a highly complex mixture of hydrocarbons. The major component hydrocarbon groupings each include compounds of a wide range of molecular weights and carbon number. Concise summaries of the toxicities of oils and their fractions are presented by Craddock (1977), Johnson (1977), Moore and Dwyer (1974), and Patten (1977), among others. Benzene, for instance, and other low boiling point components are known to be acutely and chronically toxic in organisms. A recent report by Holliday, Engelhardt et al. (1979) has summarized the toxicity effects of benzene. Naphthalenic fractions are also toxic and are readily assimilated into tissues (Blumer et al. 1970, Stegeman and Teal 1973). The paraffinic compounds are generally less reactive, and thus an oil with a larger proportion of these saturated aliphatics is also less toxic.

Midale crude oil is a petroleum from Saskatchewan. Although not Arctic in origin, this oil does resemble arctic oils. The records describe it to be similar to Prudhoe Bay oil in some respects, such as API density and proportion of paraffins. Prudhoe Bay crude is less viscous, however (Thompson et al. 1971, Coleman et al. 1973).

Once oil is exposed to air and water, rapid compositional changes usually occur. The process of aging and weathering of crude oils in and on seawater has been well documented by Boylan and Tripp (1971), Smith and MacIntyre (1971), Erhardt and Blumer (1972), Harrison et al. (1975), and Engelhardt (1978), among others. Losses of benzene compounds, and other low boiling point components, occur easily by volatilization to the air; those hydrocarbons of a more polar nature and more water soluble are lost to this medium. In a light to medium weight arctic petroleum, such as Norman Wells crude, major losses may occur within 24 hours (Engelhardt 1978). Heavier oils such as Midale crude may be expected to show less of a weathering effect.

Analysis of petroleum hydrocarbons presents a problem since no single method of extraction and identification exists which meets all requirements of cost effectiveness, sensitivity, and specificity. Many different methods of extraction are possible (Anderson et al. 1974), as well as many methods of identification (National Academy of Sciences 1975). The specificity and sensitivity of a gas chromatographic technique may be augmented by UV-absorption and UV-fluorescence analyses (Frank 1975, 1978; Wakeham 1976; Zitko and Carson 1970). The UV-absorption detection method tends to be more suitable for rapid qualitative assessments, while that of UV-fluorescence is both specific and sensitive, as well as unmodified by weathering effects (Frank 1975, 1978). This latter method was chosen to analyze the biological samples generated in this study as supplemented by gas chromatographic analysis.

5.1.3 Accumulation and Clearance

Quantification of accumulation and release of oil hydrocarbons is of use to identify specific toxicity effects with tissue levels of the oil. In a study on ringed seals, for instance, the identification of petroleum-derived hydrocarbons in liver and kidney tissues of exposed animals (Engelhardt et al. 1977, Engelhardt 1978) correlates with the low level pathology noted in these tissues (Smith and Geraci 1975, Geraci and Smith 1976). Similarly, residues of oil in brain tissues correlated with the neurological symptoms observed early in the exposure of the seals. Clearance parameters can also be determined by measuring petroleum residues in tissues and fluids. Engelhardt et al (1977) and Engelhardt (1978) indicated a rapid uptake in seals, followed by an effective clearance from body tissues resulting from metabolic activity, converting ingested oil hydrocarbons to water soluble fractions which could be excreted by way of bile and urine. One tissue which did not show a rapid clearance of hydrocarbons, but rather accumulated it, was blubber. Recognition of such storage capabilities in fatty tissues points out the possibility of a long-term chronic release from these sites, or perhaps a burst release when the contaminated animal is in need of stored fatty energy reserves. That such energy needs would occur at physiologically critical times in the life of the animal makes the release of oil from fat a particular consideration.

None of these toxicity, clearance, and storage relationships can be recognized without clear knowledge of hydrocarbon levels in the organism. Accumulation and release of Midale oil hydrocarbons were assessed in the oil-exposed polar bears.

5.2 Materials and Methods

5.2.1 Characterization of Midale Crude

5.2.1.1 Past Data Records

The petroleum oil used in this study was a medium weight crude of Saskatchewan origin, identified as a Midale crude oil. Although no recent industrial characterization of this oil, and specifically of the lot used in this study, was available, records were present in my file on older lots of Midale crude used in previous studies. These data had been supplied through the courtesy of the Shell Oil Refinery Laboratory at Sarnia and the Department of Energy, Mines and Resources Canada. These data are summarized in Tables 5-1, 5-2, and 5-3. Further characterization data was available from a 1976 assay of the oil, carried out by gas chromatography in the laboratory of F.R. Engelhardt (Figure 5-1). Although these past records were and are useful in interpreting the toxicities of the crude oil, it was necessary to assay the oil as used in this experiment for purposes of identifying particular toxic fractions, as well as the effects of weathering on these fractions and the general composition of the oil. The chemical analyses were also necessary in order to fingerprint the oil in body fluids and excretion products of the treated polar bears, identifying toxic effects with the presence of oil.

5.2.1.2 Assay Methods for Oil Characterization

Gas chromatography - Two methodologies require description. In the first instance, the gas chromatographic analysis on the 1976 sample of Midale crude requires description to allow effective comparison. Barrel-fresh crude oil was analyzed using a Varian-Aerograph (Model 2000) gas chromatograph with flame ionization detectors. Aliquots of the oil were injected in CS_2 and analyzed on a support-coated open-tubular (SCOT) OV-1 column, temperature programmed at 4°/min from 60 to 275°C, and isothermal at 275°C.

The oil used specifically in the study of this report was analyzed using a Hewlett-Packard (HP 5880 A) gas chromatograph with flame ionization detectors, equipped with a 4 foot long, 1/8 inch diameter glass column. The column packing used was 4% SE 30/6% QF 1 on chromsorb WHP 80/100, run at a temperature program of 10°C/min from 70 to 225°C, then isothermal. Aliquots of petroleum, or of organic extracts (hexane-dichloromethane, 5/2) of oil-contaminated samples, were injected with dichloromethane. All solvents used in these assays were of chromatographic grade.

Qualitative and quantitative assessment of the chromatograms was made possible by the assay of a number of hydrocarbon standards analyzed under identical conditions.

Table 5-1. Physical properties of Midale crude oil, summarized from laboratory reports from Shell Oil Refinery Laboratory at Sarnia, Ontario[a] and from the Department of Energy, Mines and Resources Canada[b], showing ranges of values for three oil assays when applicable.

ASSAY	VALUE
Colour	brownish-black to black
API density, 60°F	27.5 - 37.2
Specific gravity	0.890
Saybolt Universal Viscosity	
at 77°F, sec	95
at 100°F, sec	86
Pour point, °F	27.5
BS & W, % vol	0.10 - 0.15
Sulfur, % w	0.58 - 2.60
Carbon residue, % w	6.9 - 11.3

a two assays of Midale crude composite from 1971-72.

b one assay of Midale crude from well in 1954.

Table 5-2. Laboratory distillation yields of Midale crude oil, summarized from laboratory reports from Shell Oil Refinery Laboratory at Sarnia, Ontario[a] and from the Department of Energy, Mines and Resources Canada[b].

Source a		Source b	
Fraction (°F)	Yield (% Vol. crude)	Fraction (°F)	Yield (% Vol. crude)
IBP - 113	4.2, 7.1	81 - 122	2.0
113 - 220	8.7, 9.3	122 - 167	2.3
220 - 260	3.9, 4.4	167 - 212	3.6
260 - 315	5.0, 6.6	212 - 257	4.5
315 - 390	6.2, 8.1	257 - 302	3.8
390 - 450	5.6, 6.2	302 - 347	3.8
450 - 500	5.1, 5.7	347 - 392	3.7
500 - 550	4.6, 5.2	392 - 437	3.9
550 - 650	9.9, 10.2	437 - 482	4.6
650 - 750	4.8, 5.9	482 - 527	6.0
750 - 900	14.8, 13.0	cont. at 40 mm Hg press.	
900 - 1000	9.8, 7.0	- 392	4.0
vacuum-flashed residue	17.4, 11.3	392 - 437	4.0
		437 - 482	5.0
		482 - 527	5.4
		527 - 572	6.6
		residuum	

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[a] two assays of Midale crude composite from 1971-72

[b] one assay of Midale crude from well in 1954

Table 5-3 Composition of hydrocarbons in Midale crude oil fractions, summarized from laboratory reports from Shell Oil Refinery Laboratory at Sarnia, Ontario[a].

	Fraction Type (°F)					
Component	(IBP - 113)	S.R. Gasoline (113 - 220)	(220 - 260)	(260 - 315)	Mineral Spirits (315 - 390)	Turbine Fuel Distillate (390 - 500)
methane						
ethane	0.4, 0.8	0.2				
propane	11.2, 17.5	0.3, 0.1				
i-butane	6.5, 4.5	0.5, 0.1				
n-butane	24.0, 20.9	2.8, 2.0				
cyclopentane	1.1, 1.1	1.1, 0.9				
i-pentane	15.3, 15.1	5.5, 3.7				
n-pentane	16.9, 19.0	8.1, 6.8				
C_5 +	24.6					
2,2-di Me C_4	0.2	0.1, 0.2				
2,3-di Me C_4	6.2	7.1, 7.1				
2-Me C_5						
3-Me C_5	2.8	4.7, 4.0				
cyclopentane						
n-hexane	5.9	11.2, 13.5				
2,2-di Me C_5	0.1	0.3, 0.5				
2,4-di Me C_5						
Me Cy C_5	2.3	7.3, 4.1				
2-Me C_6						
2,3-di Me C_5						
cyclohexane	0.6	5.2, 4.7				
n-heptane						
C_6+	3.0	46.8, 52.3				
Total	100.0, 100.0	100.0, 100.0				

Table 5-3 (Continued)

	Fraction Type (°F)					
Component	(IBP - 113)	S.R. Gasoline (113 - 220)	(220 - 260)	(260 - 315)	Mineral Spirits (315 - 390)	Turbine Fuel Distillate (390 - 500)
Total paraffins			32.3, 44.8	31.4, 48.9	22.5, 37.5	
Total naphthenes			55.1, 48.5	51.3, 38.4	57.8, 44.0	
Total aromatics			12.6, 6.7	17.3, 12.7	19.7, 18.5	
Total		100.0, 100.0,	100.0, 100.0	100.0, 100.0	100.0, 100.0	

a two assays of Midale crude composite from 1971-72.

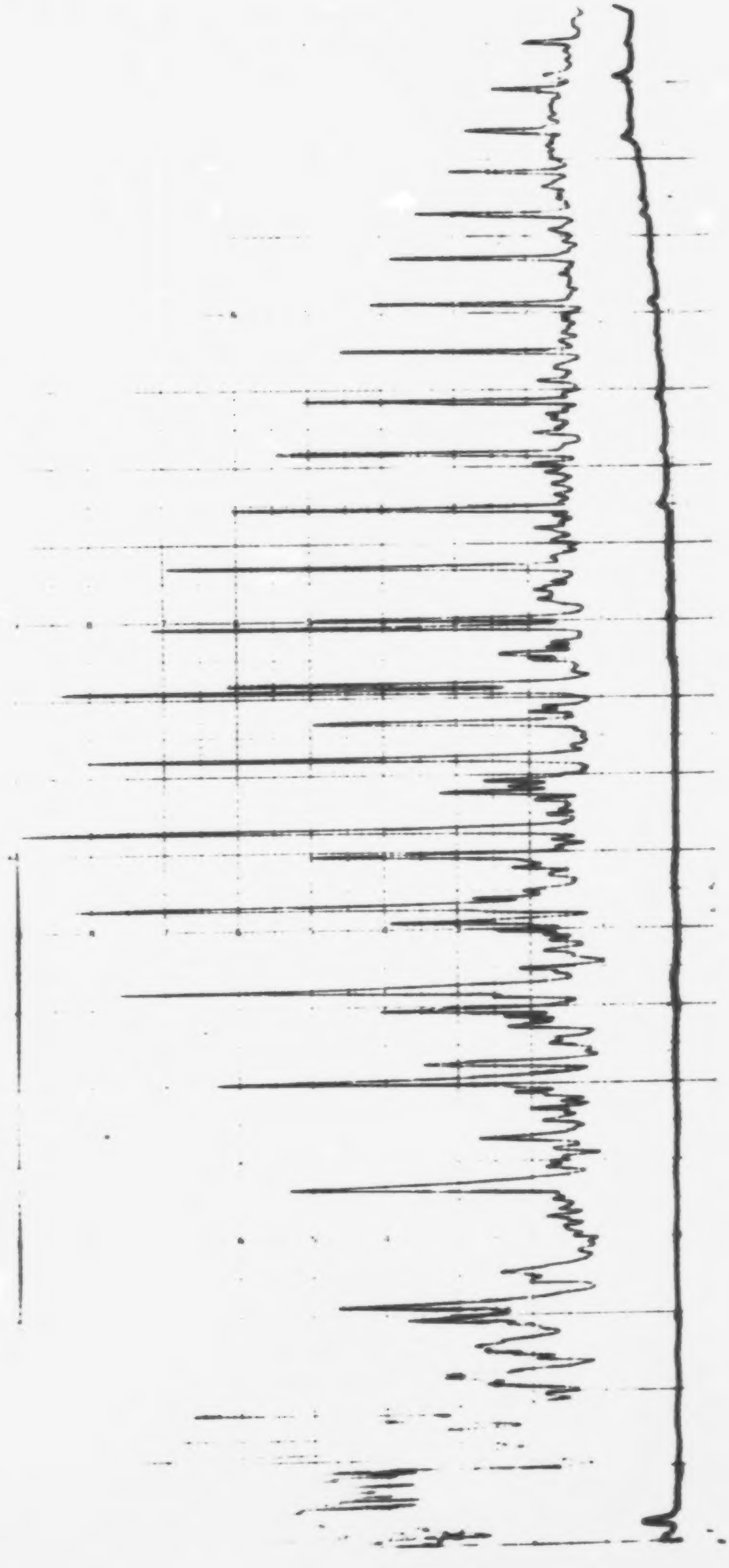

Figure 5.1. Gas chromatogram of Midale crude oil, 1976 sample.

The following standards were used: aromatics - benzene, naphthalene, phenanthrene, benzanthracene, and benzo(a)pyrene; methylated aromatics - methyl benzene (toluene), dimethyl benzene (xylenes), 1,3,5-trimethylbenzene, 1,2,3-trimethylbenzene, 1,2,4,5-tetramethyl benzene (isodurene), 2-methylnaphthalene, 1-methylnaphthalene, 2,6-dimethylnaphthalene, and 2,3-dimethylnaphthalene; aliphatics - pentane, hexane, heptane, C_{14}-paraffin, C_{15}-paraffin, and C_{16}-paraffin. Of these standards, pentane, hexane, and heptane could not be identified since they resolved with the solvent front. Thus this chromatographic assay of Midale crude oil differentiated hydrocarbon fractions starting with benzene. More volatile fractions were not resolved. It was possible to identify n-paraffins from C_{10} to C_{30}, based on the regular pattern in retention times, in reference to the C_{14} to C_{16} standards.

Fluorometry - Since it was not practically possible to analyze the many samples necessary to be analyzed for oil residues by gas chromatography, a limitation imposed by cost and time considerations, it was decided to carry out these quantitative analyses by fluorometry on a Turner spectrofluorometer (Model 430). A method modified from that of Engelhardt et al. (1977) was used. Wavelengths of excitation of emission to be used for analysis were determined by spectrofluorometric scans at 5 nm intervals of Midale crude oil dissolved in hexane-dichloromethane, 5/2, against a solvent blank. All solvents were spectroanalyzed or ultra-pure grade. This resulted in qualitatively characteristic spectra for the crude oil, with defined excitation and emission peaks.

Solvent extracts of samples from the bears were then analyzed using 310 nm excitation and 410 nm emission, with a 15 nm band width. These wavelengths were chosen since they optimized accuracy and sensitivity. The fluorescence characteristics of the crude oil showed peaks near these wavelengths, which had additionally a minimum of interference by the native fluorescence of the biological samples. Calibrations were carried out at these wavelengths using crude oil standards. Only the linear portions of the calibrations were used in the analysis, in order to improve quantitative accuracy. An effective calibration range of 0.2 to 30 ul/l was utilized. The linearity requirement needed extensive dilution of the more concentrated samples. All analyses were carried out against a solvent blank. Absolute sensitivity limits were found to be 0.01 ul/l crude oil in solvent. These could, of course, not be approached because of the native fluorescence of control samples, from non-exposed bears, or bears pre-exposure.

In all cases, the fluorescence of a specific sample type attributable to oil at the given analytical wavelengths was taken to be that fluorescence recorded to be above the mean fluorescence of all type control samples plus/minus 1 standard deviation. In terms of sensitivity, this meant that the detection limits for the various sample types were as follows (in l oil/l or kg sample):

plasma	0.20	
urine	2.8	
feces	220	
liver	0.90	
kidney	0.40	
skeletal muscle	0.50	
heart muscle	0.50	
brain	0.40	
spleen	0.50	
fat	6.7	
marrow	2.0	(estimated)
lung	0.35	
bile	100	(estimated)
stomach contents	10	(estimated)

Samples such as feces and bile were particularly high in background, or native, fluorescence.

A test was carried out to assess the relevance of using a crude oil standard, when the biological samples are likely to have residues modified by weathering and other aging factors. It was found that weathering of crude oil for 48 hours on water and in air had little qualitative or quantitative effect on the spectrofluorogram; thus, weathering of the Midale crude had little effect on the fluorometric assay methodology.

UV-absorption spectroscopy - In order to further verify that the above-background fluorescence noted in the oil-contaminated samples was attributable to Midale crude residues, UV-absorption spectral scans were carried out for each extracted sample, both from control bears and oil-exposed bears. The spectral difference between these two, for a given sample type, was then compared to UV-absorption scans of the Midale crude (10 ul/l in hexanedichloromethane).

Absorption was measured over a range of 190 - 310 nm, using a scanning spectrophotometer (Unicam SP-1800) at a band width of 1 nm and scanning speed of 2.0 nm/sec. A solvent blank was used as reference.

A qualitative assessment of the absorption spectrum was made to verify that the sample was indeed oil-contaminated. The greater difficulty of quantification, however, and decreased sensitivity of this method precluded its use as a quantitative technique in this study.

5.2.2. Dosing Protocol

The details of the experimental protocol are described by Øritsland *et al*. in Chapter 3 of this report and will not be repeated here.

The following observations made by Øritsland et al. are particularly relevant in assessing oil uptake and effects parameters. Coating with the crude oil after entry into the slick-covered pool was rapid and extensive, even at the short exposure time of Bear 2. Major loading of the fur would appear to have been general. From calculations based on a loss factor of the oil from the pool, Bear 2 was estimated to have come out of the pool with 23 l of oil in its fur. In the case of Bear 4, the calculated loading figure was 28 l after dosing. Estimations of oil loads remaining in the fur for the rest of the experimental period became exceedingly more difficult and vague. It is certain, however, that all three oiled bears carried a fur load of some litres even after several days and cleaning trials had passed.

The heavy and extended fur loading with oil is of particular import in this study. Almost immediately after leaving the dosing pool, the bears commenced to actively groom themselves, at a marked increase in time and frequency. The effect was a licking and ingestion of the oil, eventually leading to vomiting and diarrhea. The oil was also spread further over and into the pelt of the animal. Although three days after immersion, a sawdust and snow litter effected external peripheral cleaning, it is unlikely that the bear would have lost much of the deeper oil coating next to its skin. An interesting aside here is that all of the oiled bears also licked oil off the structural components of their cages. Thus, although it remains impossible to assess an exact dosage of oil to the bears because neither the experimental design nor circumstances permitted this, the bears can be expected to have ingested oil after immersion, probably of the order of litres. It may also be expected that an effective exposure of each oiled bear continued for the duration of the study in that the animals continued to groom, presumably taking in at least some of the deeper layers of oil investing the fur. These quantities are even more difficult to estimate.

5.3 Results

5.3.1 Characteristics of Midale Crude

5.3.1.1 Resolution of Hydrocarbons by Gas Chromatography

A chromatographic separation of hydrocarbon fractions in Midale crude oil, as added to the pool in a 1 cm slick 36 hours prior to immersion of the bears, showed the typically complex pattern of any crude oil (Figure 5-2). A large volume proportion of the oil was voided in the assay at short retention times (RT), less than two and one-half minutes. Resolution at less than one minute RT, however, was particularly poor. A regular pattern of major peaks resolved at graded intervals starting at about two and one-half minutes (e.g. 2.67, 4.27, 6.19, 7.77 etc.). These paraffin compounds served as ready landmarks to identify empirically some other

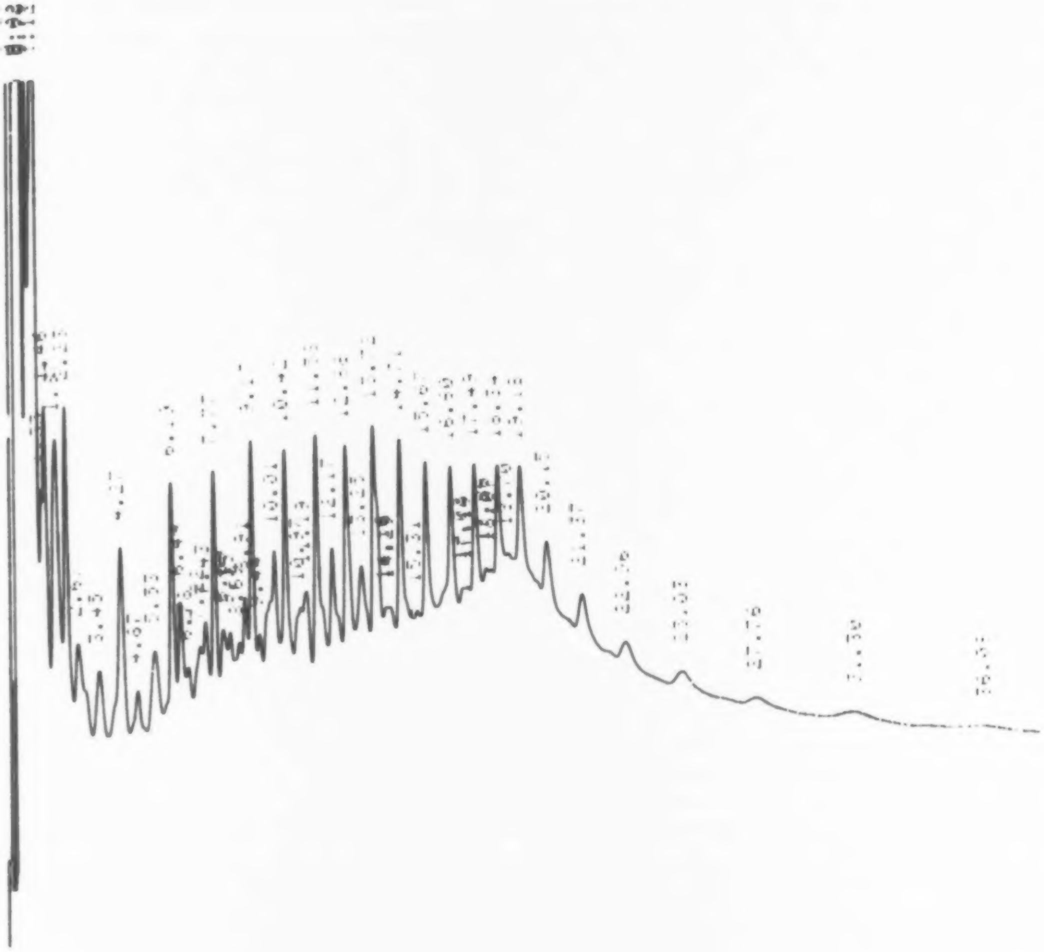

Figure 5-2. Gas chromatogram of unmodified Midale crude oil.

peaks. Aromatic hydrocarbons, found between the paraffin peaks, were evident. These included hydrocarbons of the naphthalenic series in a region from perhaps 6 to 14 minutes RT. Methylated aromatics were also found in this region.

While such qualitative assessment of the oil chromatogram did yield significant information, it was necessary to identify positively, by using established standards, at least a number of compounds. This allowed identification and quantification of peaks, such as shown in Figure 5-2, by their RT values and proportional areas (Table 5-4). Seventeen of the 20 standards tested which could be resolved are presented in Table 5-5, showing RT and concentration values used for calculations of chromatograms derived from oil and biological samples in this study. The standards pentane, hexane, and heptane eluted with the dichloromethane solvent front.

The unmodified Midale crude oil showed a relatively high percentage of benzenes, 9% by volume based on the three standards alone. It should be noted that the "benzene" peak at RT 0.37 min undoubtedly includes other low boiling point hydrocarbons, and thus is not specific. A large proportion of naphthalenes, including naphthalene and various methylated naphthalenes, was present in the crude oil. The few standards alone accounted for nearly 2% by volume. Naphthalene was present at 0.7% by volume. The chromatogram indicated that the total naphthalene proportion was probably several factors greater than that indicated by the standards. The paraffins accounted for a sizeable percentage of the volume of the oil, increasing in their relative proportion with increasing carbon chain length to C_{30}. A very rough estimate based on the use of three standards, their peak areas, and their RT values suggests that total paraffins may account for perhaps 50% of the volume of the crude oil.

5.3.1.2 Spectral Characteristics

Fluorescence spectrographs of the Midale crude oil revealed well defined excitation and emission peaks (Figure 5-3). The excitation scan had two peaks, one of 80% maximum excitation magnitude at 300 nm, and the other at 355 nm. Excitation sensitivity decreased rapidly thereafter. Emission scans were also biphasic, one peak at 365 nm, also about 80% in magnitude of the second peak, at 410 nm. In order to minimize overlap, and to improve sensitivity in the hydrocarbon residue assay, excitation near the 300 nm peak and emission at 410 nm were used.

The UV-absorption spectrum for Midale crude was found to be well defined, with a broad major peak about 233 nm (Figure 5-4). Secondary peaks, as shoulders on the large peak, occurred at 217 nm and at 262 nm. The characteristic UV-spectral absorption pattern allowed identification of the existence of oil residues in biological samples. Plasma from

Table 5-4 Quantification of gas chromatogram of unmodified Midale crude oil.

RT	AREA%	n/or ug/peak	% vol.	Compound
0.33	6.699	0.818	2.15	benzene
0.62	3.697	1.18	3.10	methylbenzene
0.79	1.256			
1.12	2.867	1.07	2.81	dimethylbenzene
1.33	0.414			
1.46	1.041			
1.87	1.550			
2.23	1.284			C_{10} paraffin
2.67	1.375			
3.45	1.053			
4.27	1.738			C_{11} paraffin
4.87	1.114	0.254	0.670	1,2,4,5-tetramethylbenzene
5.53	1.200			
6.19	1.358			C_{12} paraffin
6.49	0.823	0.270	0.711	naphthalene
6.78	0.530			
7.22	0.811			
7.43	0.722			
7.77	1.029			C_{13} paraffin
8.09	0.729	0.346	0.912	2-methylnaphthalene
8.35	0.584	0.467	1.23	1-methylnaphthalene
8.69	0.696			
8.91	0.641			
9.17	1.067	0.642	1.70	C_{14} paraffin
9.44	0.536			
10.01	1.759			
10.42	1.520	0.894	2.35	C_{15} paraffin
10.97	0.900			
11.19	0.962			
11.58	1.798	0.933	2.46	C_{16} paraffin
12.17	1.463			
12.68	1.562			C_{17} paraffin
13.25	1.600			
13.72	1.729			C_{18} paraffin
14.16	0.504			
14.29	0.759			
14.71	2.077			C_{19} paraffin
15.31	0.898			
15.67	1.836			C_{20} paraffin
16.60	2.646			C_{21} paraffin
17.02	0.719			
17.19	0.551			
17.49	1.539			C_{22} paraffin

Table 5-4 (Continued)

RT	AREA%	n/or ug/peak	% vol.	Compound
17.88	0.747			
18.07	0.565			
18.34	1.614			C_{23} paraffin
18.70	0.857			
19.18	3.505			C_{24} paraffin
20.15	3.315			C_{25} paraffin
21.37	3.755			C_{26} paraffin
22.96	4.072			C_{27} paraffin
25.03	5.043			C_{28} paraffin
27.76	5.307			C_{29} paraffin
31.30	7.362			C_{30} paraffin
36.08	4.824			

Table 5-5. Characteristics of selected hydrocarbon standards as assessed by gas chromatography. Compounds represented in parentheses are extrapolated.

Compound Category			Retention times (±0.08 min.)	Peak Area/nl or g
Aromatics	Methylated Aromatic	Paraffins		
benzene			0.37	60,200
	methyl benzene (toluene)		0.63	23,617
	dimethyl benzene (xylene)		1.10	19,149
	1,3,5-trimethyl benzene		2.02	21,272
		(C_{10})	(2.25)	
	1,2,3-trimethyl benzene		2.98	24,480
		(C_{11})	(4.30)	
	1,2,4,5-tetramethyl benzene		4.81	31,221
		(C_{12})	(6.20)	
naphthalene			6.43	21,741
		(C_{13})	(7.79)	
	2-methylnaphthalene		8.12	15,000
	1-methylnaphthalene		8.30	8,917
		C_{14}	9.15	11,773
	2,6-dimethylnaphthalene		9.68	6,329
	2,3-dimethylnaphthalene		10.11	5,332
		C_{15}	10.41	12,128
		C_{16}	11.57	13,739
		(C_{12})	(12.70)	
		(C_{18})	(13.74)	
phenanthrene			14.07	18,365
		(C_{19})	(14.72)	
		(C_{20})	(15.69)	
		(C_{21})	(16.61)	
		(C_{22})	(17.50)	
		(C_{23})	(18.35)	
		(C_{24})	(19.19)	
		(C_{25})	(20.18)	
benzanthracene			20.48	18,444
		(C_{26})	(21.40)	
		(C_{27})	(22.98)	
		(C_{28})	(25.06)	
		(C_{29})	(27.80)	
benzo(a)pyrene			28.00	18,499
		(C_{30})	(31.37)	

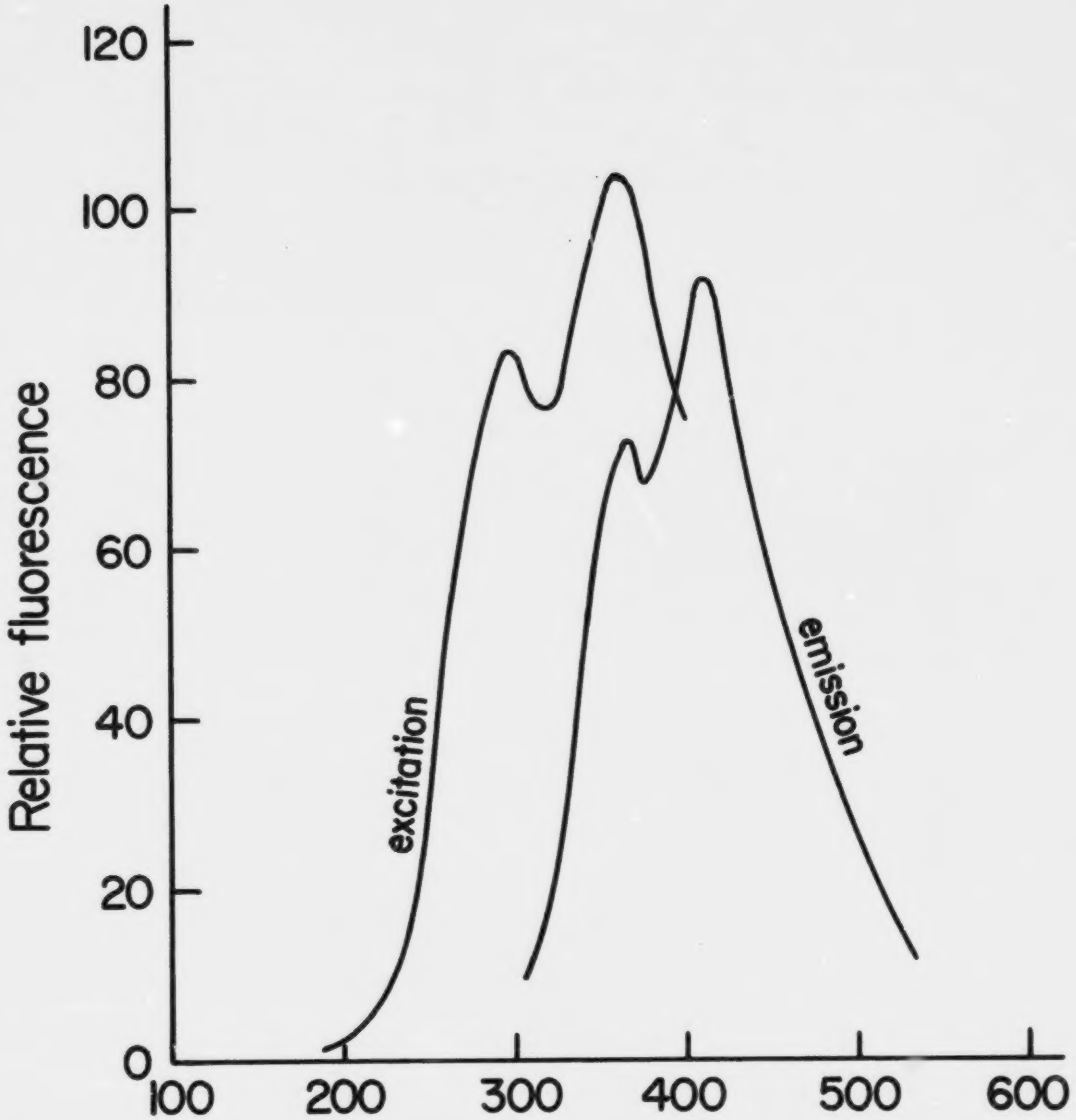

Figure 5-3. Fluorescence spectrograms of Midale crude oil.

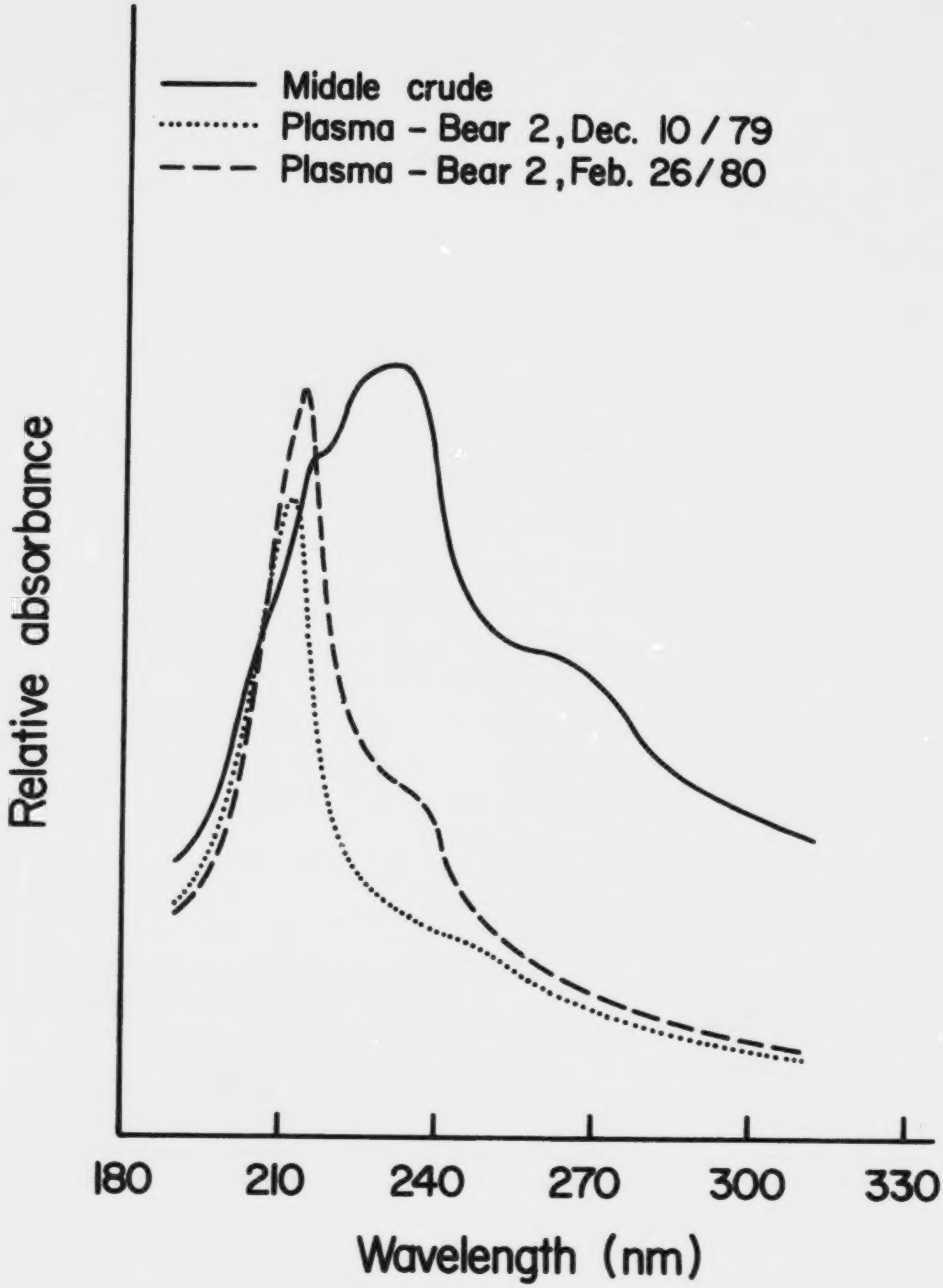

Figure 5-4. UV-absorption spectrograms of Midale crude oil and of control and oil-contaminated plasma extracts.

oil-contaminated Bear 2, for example, exhibited a shift in absorption pattern corresponding to the peaks observed in a crude oil scan. A more obvious characteristic patterning was noted in samples of urine, from Bear 2 for example (Figure 5-5). Fecal extracts from this bear at a time-high fecal oil concentration positively demonstrated the presence of oil (Figure 5-6). It must be noted that the magnitude of the absorption patterns in these figures are not directly related to each other in concentration. No allowance has been made for differences in absolute concentration in the extracts or dilutions.

5.3.2 Weathering Effects

Assessment of the chromatogram for 24-hour weathered Midale crude (Figure 5-7) showed few obvious differences from the unmodified crude (Figure 5-2). There appeared to be a decrease in the solvent front-associated envelope of very low boiling point hydrocarbons, however. More quantitatively, an enhancement of fractions between the "benzene" to the C_{15} paraffin had occurred (Table 5-6). The results indicate a relative loss, by 24 hours, of the lowest boiling point fractions (less than C_5), with a concomitant increase in fractions of somewhat higher boiling point and retention time.

By 48 hours of weathering, there has been a further partial loss of the low boiling point fractions; evident from a comparison among the chromatograms of unmodified, 24-hour weathered and 48-hour weathered (Figure 5-8) oil. No other changes are immediately evident, but a calculation of relative concentration shows enhancement of the approximately C_{10} to C_{24} range of hydrocarbons, as compared to the unmodified oil standard (Table 5-7).

5.3.3 Oil Residues in Biological Samples

5.3.3.1 Vomitus and Stomach Contents

Chromatographic assessment of organic solvent extracts of vomitus samples from Bears 2, 3 and 4, taken the day following the oil exposure, showed characteristic Midale petroleum hydrocarbon patterns (Figure 5-9). The wide spectrum of unresolved low boiling point fractions, aromatics, and paraffins was present, similar to the unmodified and weathered crude. There appears to have been an enhancement in the C_8 to C_{16} identified fractions (Tables 5-8, 5-9 and 5-10), as compared to the weathered oil to which the bears were exposed (Tables 5-6 and 5-7). This relative enhancement of higher boiling point fractions and relative loss of the low boiling point fractions extended to the larger paraffins as well, although these were not standardized.

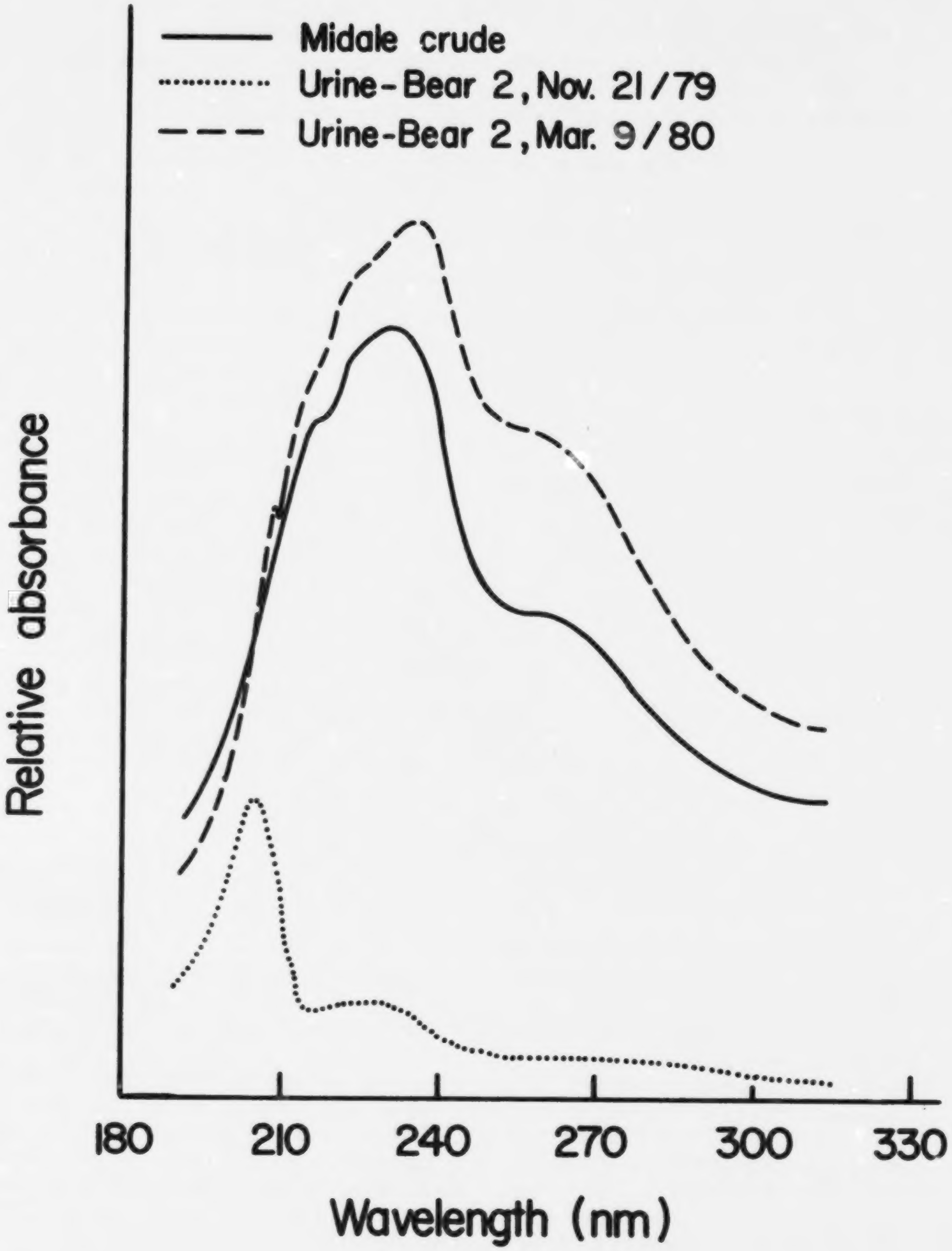

Figure 5-5. UV-absorption spectrograms of Midale crude oil and of control and oil-contaminated urine extracts.

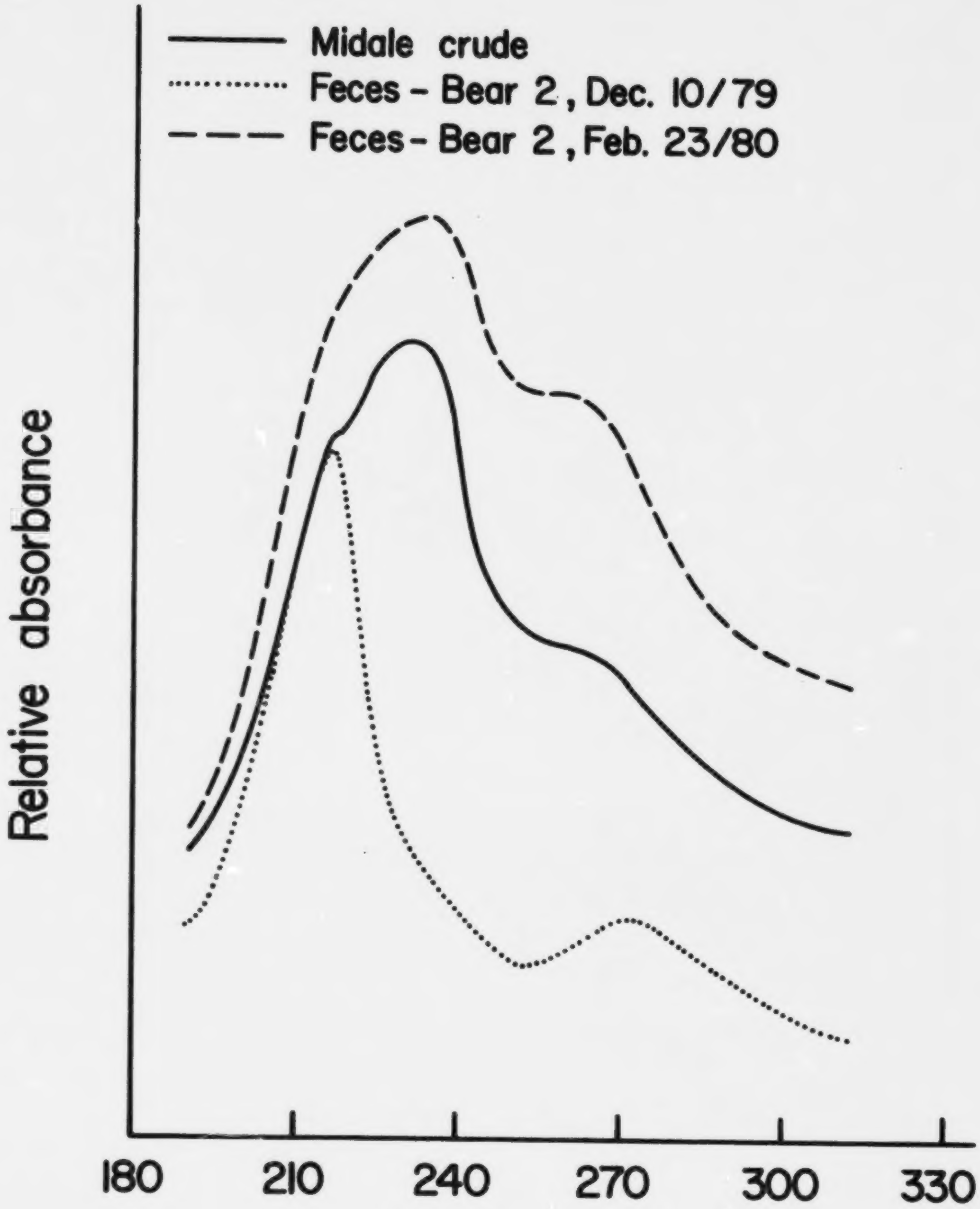

Figure 5-6. UV-absorption spectrograms of Midale crude oil and of control and oil-contaminated feces extracts.

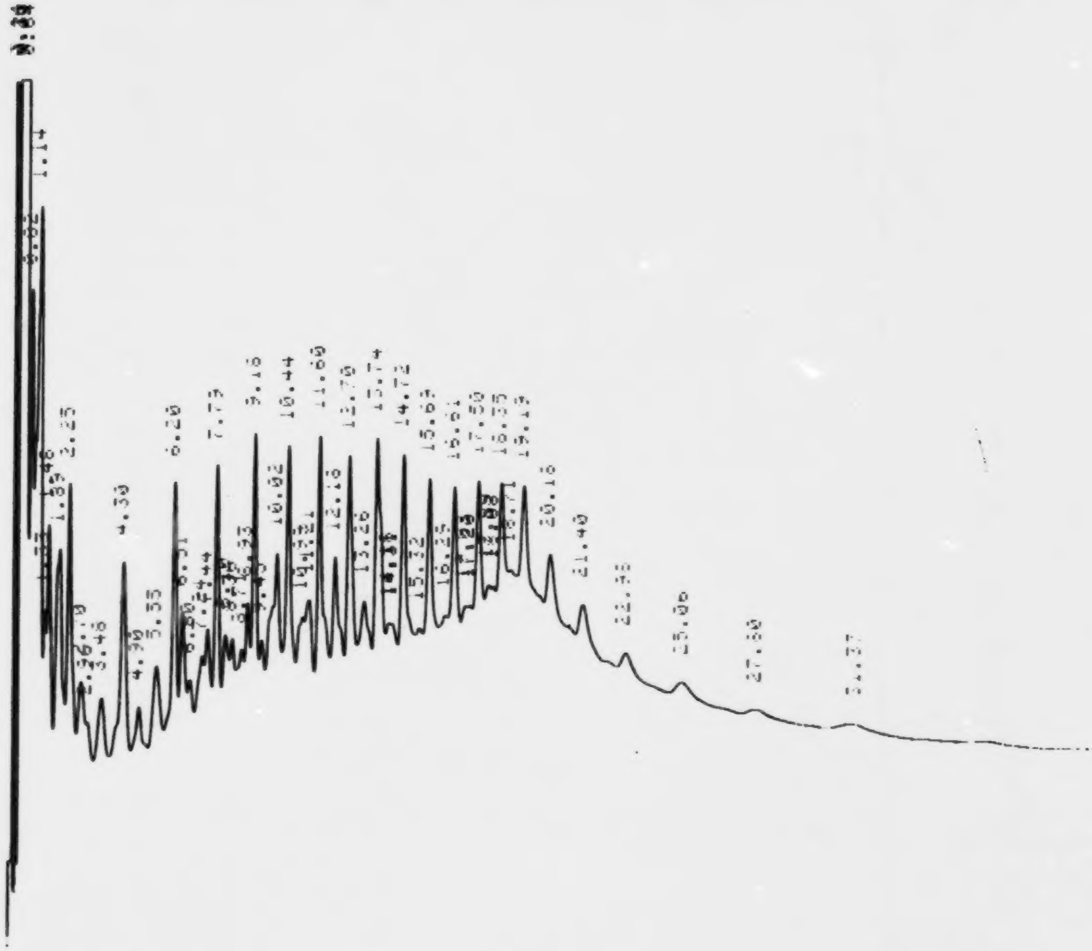

Figure 5-7. Gas chromatogram of a 24-hour weathered Midale crude oil.

Table 5-6. Quantification of gas chromatogram of 24-hour weathered Midale crude oil.

RT	AREA %	nl or μl/peak	Normalized % vol.	% oil std.	Compound
0.26	2.073				
0.36	39.133	1.79	12.2	567	benzene
0.64	3.921	0.458	3.13	101	methylbenzene
0.82	2.151			173	
1.14	4.645	0.669	4.56	163	dimethylbenzene
1.33	0.799			193	
1.46	1.605			174	
1.89	2.706			175	
2.25	2.467			192	C_{10} paraffin
2.70	1.405			102	
2.96	0.766	0.0886	0.619	+	1,2,3-trimethylbenzene
3.48	1.608			152	
4.30	2.755			159	C_{11} paraffin
4.90	1.393	0.123	2.17	124	1,2,4,5-tetramethylbenzene
5.55	1.555			130	
6.20	2.076			153	C_{12} paraffin
6.51	1.165	0.148	1.01	142	naphthalene
6.80	0.614			116	
7.24	0.972			120	
7.44	0.923			128	
7.79	1.561			152	C_{13} paraffin
8.10	0.864	0.159	1.08	126	2-methylnaphthalene
8.36	0.658	0.204	1.39	113	1-methylnaphthalene
8.71	0.736			106	
8.93	0.763			119	
9.18	1.564	0.367	2.50	147	C_{14} paraffin
9.45	0.512			96.2	
10.02	1.993			113	
10.44	1.776	0.404	2.75	117	C_{15} paraffin
10.99	0.782			87.0	
11.21	0.630			86.5	
11.60	1.764	0.354	2.41	104	C_{16} paraffin
12.18	1.073			73.6	
12.70	1.468			94.3	C_{17} paraffin
13.26	0.764			43.3	
13.74	1.563			73.5	C_{18} paraffin
14.18				37.1	
14.31				29.4	
14.72	1.129			54.5	C_{19} paraffin

Table 5-6 (Continued).

RT	AREA %	nl or µl/peak	Normalized % vol.	% oil std.	Compound
15.32	0.110			15.8	
15.69	0.844			46.0	C_{20} paraffin
16.29	0.113			+	
16.61	0.663			25.9	C_{21} paraffin
17.03	0.049			6.87	
17.20	0.036			6.59	
17.50	0.601			39.1	C_{22} paraffin
17.89	0.658			7.83	
18.08	0.029			5.22	
18.35	0.525			32.8	C_{23} paraffin
18.71	0.045			5.21	
19.19	0.550			15.9	C_{24} paraffin
20.18	0.363			11.56	C_{25} paraffin
21.40	0.245			6.55	C_{26} paraffin
22.96	0.252			3.77	C_{27} paraffin
25.06	0.102			4.02	C_{28} paraffin
27.80	0.804			1.58	C_{29} paraffin
31.37	0.997			2.42	C_{30} paraffin

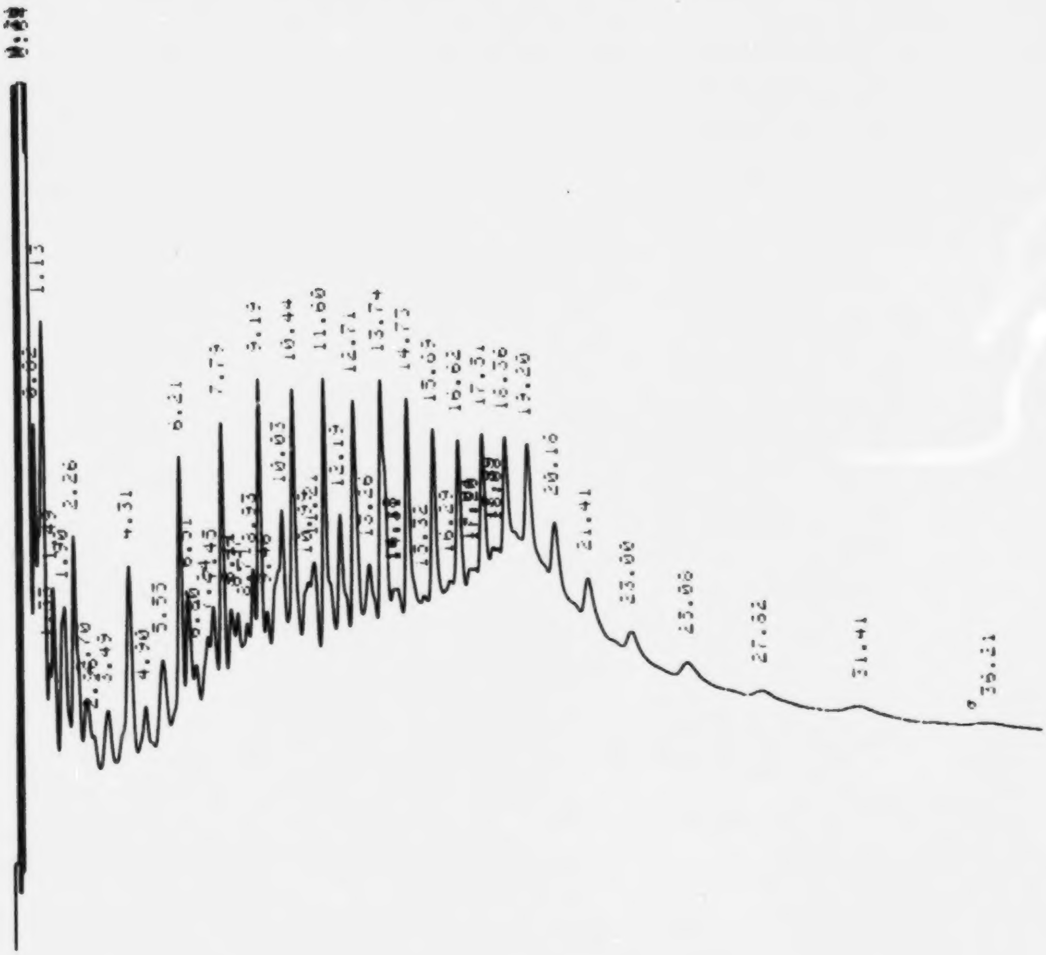

Figure 5-8. Gas chromatogram of 48-hour weathered Midale crude oil.

Table 5-7. Quantification of gas chromatogram of 48-hour weathered Midale crude oil.

RT	AREA %	nl or ug/peak	Normal- ized % vol	% oil std.	Compound
0.26	1.127				
0.38	11.195	0.921	3.60	162	benzene
0.64	1.568	0.329	1.28	40.2	methylbenzene
0.82	0.961			76.8	
1.13	2.129	0.551	2.15	74.2	dimethylbenzene
1.33	0.357			86.1	
1.49	0.907			87.1	
1.90	1.395			89.9	
2.26	1.461			114	C_{10} paraffin
2.70	0.844			61.4	
2.96	0.533	0.108	0.419	+	1,2,3-trimethylbenzene
3.49	1.146			107	
4.31	2.038			117	C_{11} paraffin
4.90	1.296	0.206	0.801	116	1,2,4,5-tetramethylbenzene
5.55	1.447			121	
6.21	1.794			132	C_{12} paraffin
6.51	1.073	0.244	0.950	130	naphthalene
6.80	0.675			127	
7.24	1.055			130	
7.45	0.962			133	
7.79	1.454			141	C_{13} paraffin
8.11	0.981	0.324	1.26	135	2-methylnaphthalene
8.37	0.777	0.431	1.68	133	1-methylnaphthalene
8.71	0.939			135	
8.93	0.881			137	
9.19	1.546	0.650	2.53	145	C_{14} paraffin
9.46	0.714			133	
10.03	2.445			139	
10.44	2.126	0.868	3.38	118	C_{15} paraffin
10.99	1.205			134	
11.21	1.275			132	
11.60	2.444	0.881	3.43	136	C_{16} paraffin
12.19	1.904			130	
12.71	2.107			138	C_{17} paraffin
13.26	1.925			120	
13.74	2.317			134	C_{18} paraffin
14.18	0.612			121	
14.31	0.925			122	
14.73	2.636			127	C_{19} paraffin

Table 5-7 (Continued).

RT	AREA %	nl or ug/peak	Normalized % vol.	% oil std.	Compound
15.32	0.845			121	
15.69	2.266			124	C_{20} paraffin
16.29	1.191			+	
16.62	1.993			75.3	C_{21} paraffin
17.04	0.628			115	
17.20	0.660			120	
17.51	1.956			127	
17.89	0.898			120	
18.08	0.640			113	
18.36	[illegible]377			209	C_{23} paraffin
19.20	[illegible]12			109	
20.18	[illegible]			111	C_{24} paraffin
21.41	3.7[illegible]			99.2	C_{25} paraffin
23.00	3.476			85.3	C_{26} paraffin
25.08	3.466			69.1	C_{27} paraffin
27.82	2.498			47.0	C_{28} paraffin
31.41	1.437			19.5	C_{29} paraffin
36.21	0.044			1.09	C_{30} paraffin

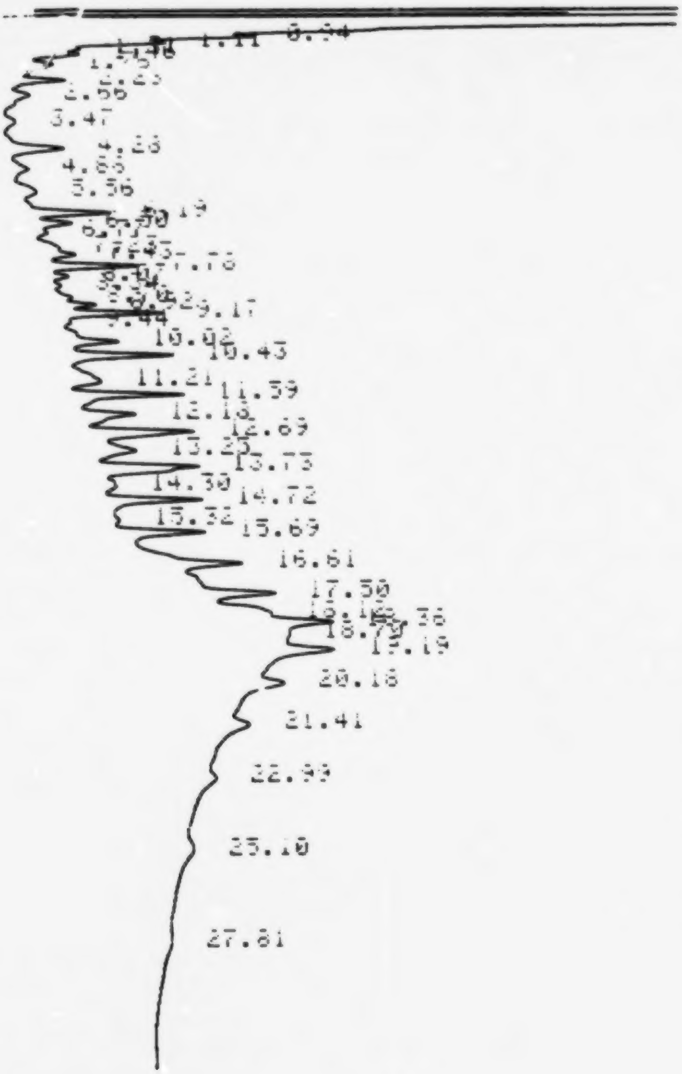

Figure 5-9. Representative gas chromatogram of Midale crude oil residues in vomitus (Bear 3).

Table 5-8. Quantification of gas chromatogram of extract of vomitus from Bear 2, Feb. 22, 1980.

RT	AREA %	nl or ug/peak	µl/or mg/g sample	Compound
0.37	21.111			
1.16	0.637	0.166	4.37	dimethylbenzene
1.32	0.330			
1.48	0.732			
1.89	1.086			
2.26	0.698			C_{10} paraffin
2.70	0.705			
2.96	0.439	0.068	1.79	1,2,3, trimethylbenzene
3.49	0.920			
4.30	1.489			C_{11} paraffin
4.90	1.031	0.125	3.29	1,2,4,5, tetramethylbenzene
5.55	1.061			
6.21	1.178			C_{12} paraffin
6.51	0.713	0.124	3.27	naphthalene
6.60	0.476			
7.24	0.726			
7.45	0.635			
7.79	0.877			C_{13} paraffin
8.10	0.640	0.161	4.24	2 methylnaphthalene
8.37	0.528	0.225	5.90	1 methylnaphthalene
8.71	0.629			
8.93	0.565			
9.19	0.910	0.292	7.71	C_{14} paraffin
9.46	0.468			
10.03	1.553			
10.45	1.341	0.418	11.0	C_{15} paraffin
10.99	0.809			
11.22	0.854			
11.60	1.577	0.435	11.4	C_{16} paraffin
12.19	1.295			
12.71	1.369			C_{17} paraffin
13.27	1.384			
13.74	1.485			C_{18} paraffin
14.19	0.449			
14.32	0.702			
14.73	1.845			C_{19} paraffin
15.33	0.646			
15.69	1.654			C_{20} paraffin
16.32	0.954			
16.62	1.495			C_{21} paraffin
17.24	1.215			

Table 5-8 (Continued).

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
17.51	1.519			C_{22} paraffin
17.92	0.741			
18.11	0.546			
18.37	1.667			C_{23} paraffin
18.77	0.645			
19.20	4.195			C_{24} paraffin
20.18	4.117			C_{25} paraffin
21.42	4.949			C_{26} paraffin
23.02	5.784			C_{27} paraffin
25.10	7.597			C_{28} paraffin
27.64	8.408			C_{29} paraffin

Table 5-9 Quantification of gas chromatogram of extract of vomitus from Bear 3, Feb. 28, 1980.

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
0.36	18.087			
0.94	0.614			
1.11	0.532	0.120	2.93	dimethylbenzene
1.31	0.258			
1.46	0.451			
1.76	0.653			
2.23	0.543			C_{10} paraffin
2.66	0.803			
3.47	0.613			
4.28	0.958			C_{11} paraffin
4.88	0.772	0.107	2.61	1,2,4,5, tetramethylbenzene
5.56	0.756			
6.19	0.918			C_{12} paraffin
6.50	0.538	0.108	2.63	naphthalene
6.77	0.386			
7.23	0.565			
7.43	0.542			
7.78	0.712			C_{13} paraffin
8.07	0.461	0.133	3.24	2 methylnaphthalene
8.34	0.400	0.194	4.74	1 methylnaphthalene
8.70	0.577			
8.92	0.493			
9.17	0.789	0.290	7.07	C_{14} paraffin
9.44	0.372			
10.02	1.291			
10.43	1.167	0.416	10.2	C_{15} paraffin
11.21	1.405			
11.59	1.362	0.429	10.5	C_{16} paraffin
12.18	1.222			
12.69	1.268			C_{17} paraffin
13.25	1.430			
13.73	1.422			C_{18} paraffin
14.30	1.099			
14.72	1.534			C_{19} paraffin
15.32	0.885			
15.69	1.537			C_{20} paraffin
16.61	2.602			C_{21} paraffin
17.50	3.302			C_{22} paraffin
18.12	1.715			
18.36	2.323			C_{23} paraffin
18.70	1.115			
19.19	5.032			C_{24} paraffin

Table 5-9 (Continued).

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
20.18	4.933			C_{25} paraffin
21.41	5.917			C_{26} paraffin
22.99	6.766			C_{27} paraffin
25.10	8.788			C_{28} paraffin
27.81	9.877			C_{29} paraffin

Table 5-10. Quantification of gas chromatogram of extract of vomitus from Bear 4, Feb. 25, 1980.

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
0.37	37.450			
1.15	0.969	0.108	2.63	dimethylbenzene
1.47	0.673			
1.76	0.676			
2.04	0.175	0.0176	0.429	1,3,5,Trimethylbenzene
2.24	0.624			C_{10} paraffin
2.67	0.953			
3.46	0.659			
4.27	1.027			C_{11} paraffin
4.87	0.679	0.0464	1.13	1,2,4,5, tetramethylbenzene
5.54	0.675			
6.18	1.018			C_{12} paraffin
6.49	0.580	0.0569	1.39	naphthalene
6.77	0.343			
7.22	0.349			
7.42	0.573			
7.77	0.902			C_{13} paraffin
8.06	0.463	0.0658	1.60	2 methylnaphthalene
8.34	0.373	0.0893	2.18	1 methylnaphthalene
8.70	0.567			
8.91	0.555			
9.17	1.019	0.184	4.50	C_{14} paraffin
9.44	0.362			
10.02	1.409			
10.43	1.422	0.250	6.10	C_{15} paraffin
11.21	1.547			
11.59	1.656	0.257	6.27	C_{16} paraffin
12.18	1.346			
12.70	1.575			C_{17} paraffin
13.25	1.249			
13.74	1.591			C_{18} paraffin
14.31	0.877			
14.73	1.602			C_{19} paraffin
15.33	0.632			
15.70	1.471			C_{20} paraffin
16.34	1.671			
16.62	1.729			C_{21} paraffin
17.06	0.572			
17.24	0.572			
17.52	1.445			C_{22} paraffin
17.90	1.810			
18.09	0.828			

Table 5-10 (Continued).

RT	AREA %	nl or ug/peak	µl or mg/g sample	Compound
18.37	2.633			C_{23} paraffin
18.71	0.984			
19.21	5.466			C_{24} paraffin
20.20	4.491			C_{25} paraffin
21.44	4.275			C_{26} paraffin
23.03	5.249			C_{27} paraffin
25.13	1.650			C_{28} paraffin
27.66	0.034			C_{29} paraffin
31.40	0.033			C_{30} paraffin

Fluorometric analysis showed the vomitus samples to contain between 52 and 60% volume/weight of crude oil (Table 5-11). This correlated with the extensive grooming of the fur, observed in all bears after oil immersion.

Even up to five weeks after the initial oil exposure, there was evidence of oil in stomach contents (Table 5-11). The oil levels in the stomach contents of Bears 3 and 4 were appreciable, at 0.176 and 37.0 ml/kg, respectively. Fur was also found in the stomach contents, suggesting grooming activity.

5.3.3.2 Fur

One sample of fur was analyzed, clipped at skin level from Bear 2 on April 4. The fur was found to be slightly discoloured with a yellowish-brown tinge, and, at the base of the hairs, matted with a blackish, highly viscous substance. Following organic solvent extraction, all of the discolouration and matting substance was removed and the fur became white in colour.

Chromatographic analysis indicated the presence of petroleum (Figure 5-10). No trace of hydrocarbons less than C_{13} could be detected, whereas a relative enhancement of the larger molecular weight was obvious (Table 5-12).

Fluorometric analysis showed that the fur, on a per weight basis before extraction, had 34% oil residues. On a clean dry weight basis this represents about a 50% weight increase. The matting and discolouring substance was undoubtedly a petroleum residue.

5.3.3.3 Plasma

Evidence of the uptake of Midale hydrocarbons into the system of the bears is seen in plasma (Table 5-13). Maximum oil concentrations were noted in plasma in samples taken soon after the oiling event, that is, within the first week. Concentrations from 31 to 36μl/l were found. These decreased in the course of the following four to five weeks to a level below the detection limit in Bear 3 and approximately 3μl/l in Bears 2 and 4. Bear 2 showed an interesting and marked rise in plasma oil in the April 14 sample. The significance of this increase coinciding with a concerted mechanical cleaning episode in the bear is discussed subsequently. Plasma oil in Bear 2 decreased to near trace levels in July.

Note should be taken of the apparent low levels of oil, as determined by the fluoroassay, in plasma samples taken at or soon after capture in Bears 1, 2, and 3. This suggests some exposure or contamination of these "nuisance" bears in the wild.

Table 5-11. Residues of Midale crude oil in samples of vomitus and stomach contents, taken after oil exposure.

		Concentration (ml/kg)		
Specimen	Date	Bear 2	Bear 3	Bear 4
Vomitus	Feb. 22	520		
	Feb. 28		575	
	Feb. 25			600
	Feb. 26			605
Stomach Contents	Mar. 21			37.0
	Mar. 25		0.176	

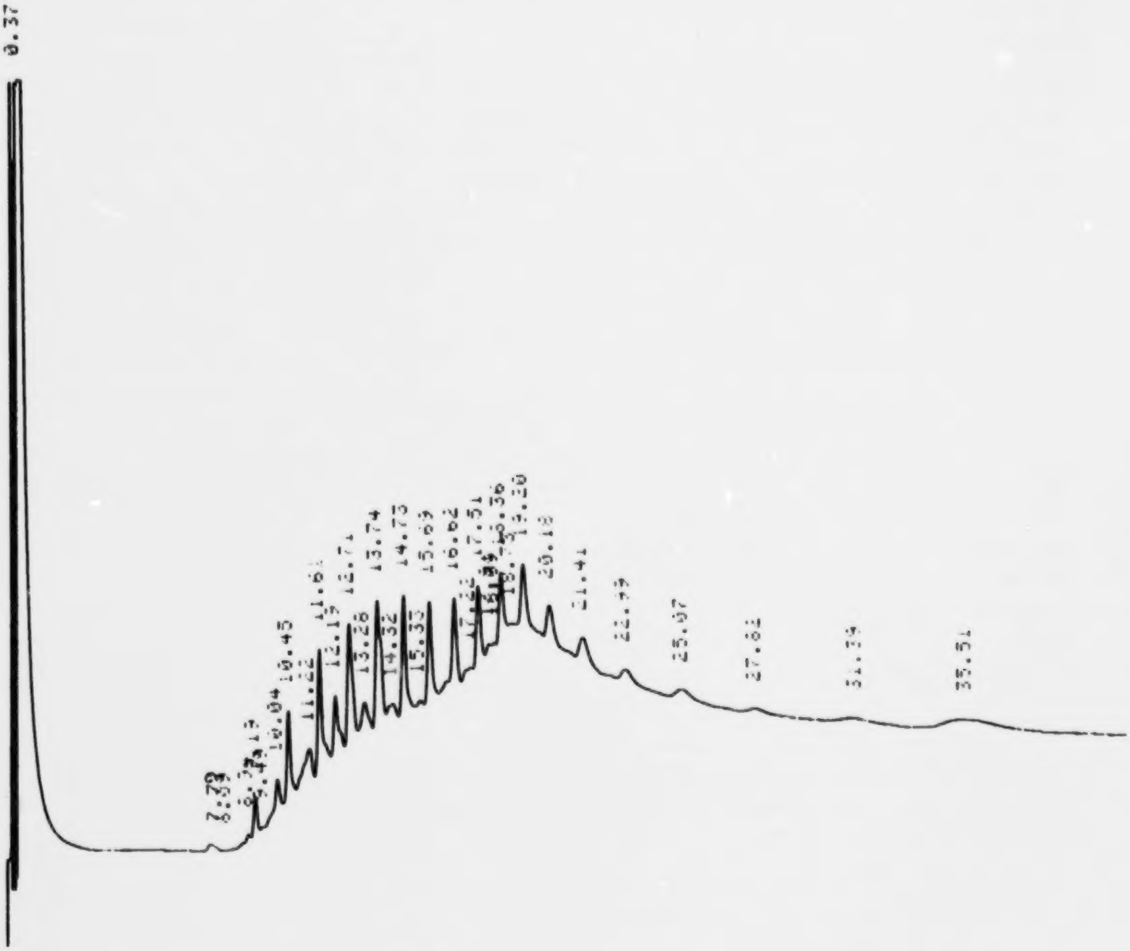

Figure 5-10. Gas chromatogram of fur extract of Bear 2, April 4, 1980.

5.3.3.4 Tissues

Midale crude oil residues were found in an array of tissues taken from Bears 3 and 4 on March 25 and March 21, respectively, during the post-mortem necropsy. Particularly high residue levels were found in bone marrow, brain, and kidney tissues, although not consistently between the two bears. Liver, lung, and skeletal and heart muscle ranked next in descending order of concentration. Both subcutaneous and mesenteric fat were remarkable in showing no residues present, to the limits of detectability (Table 5-14).

5.3.3.5 Urine

Urine samples, taken as available from the bears, showed no oil-related fluorescence when collected before the oil exposure and marked activity after oiling (Table 5-15). Urine had oil levels as high as 714 µl/l (Bear 2). There was oil present in urine for the duration of the experimental period in Churchill. A point of relevance here is that only those urine samples identified with a time of day in hours, as well as the March 9 sample taken directly during the cleaning of Bear 2, can be considered as fresh and presumably uncontaminated by extraneous oil picked up after standing in the collecting pan for an unspecified length of time. Nonetheless, even these fresh samples showed marked oil residues, including the 714 µl/l maximum in Bear 2, and levels of 27 to 42 µl/l three to four weeks after exposure.

Urine was also analyzed chromatographically. Although the quality of the resolution was very poor and thus is not presented here for quantitative analysis, it was obvious from the chromatograms, taken from extracts of fresh urine samples, that Midale crude residues were present. A characteristic spacing of paraffin peaks was evident.

5.3.3.6 Feces and Bile

The chromatographic pattern of fecal samples shows that petroleum hydrocarbons were present, of the Midale oil type (Figure 5-11). A decrease in the relative proportion of low boiling point fractions can be visualized. More specifically, Tables 5-16, 5-17, and 5-18 quantify an increase in all standards above C_8 compounds, including polymethylated benzenes, naphthalenes, and paraffinic hydrocarbons. This occurred in fecal samples from all three oiled bears taken within one to two days after the oil immersion.

Total oil concentrations in feces are high soon after exposure (Table 5-19), with maximum concentrations as much as 90, 60 and 46% volume/weight feces in Bears 2, 3 and 4, respectively. Fecal residues were recorded for the duration of the post-oil observation period, although there was a general decrease with time post-exposure. An anomaly in this was Bear 2 on March 22 and April 4, when the animal again had

Table 5-12. Quantification of gas chromatogram of fur extract from Bear 2, April 4, 1980.

RT	AREA %	nl or ug/peak	µl or mg/g sample	Compound
0.37	33.132			
7.79	0.009			C_{13} paraffin
8.09	0.001			
8.94	0.097			
9.19	0.273	0.0560	0.708	C_{14} paraffin
9.49	0.089			
10.04	0.696	0.315	3.97	2,3 dimethylnaphthalene
10.45	1.076	0.214	2.71	C_{15} paraffin
11.22	1.447			
11.61	1.813	0.319	4.03	C_{16} paraffin
12.19	1.611			
12.71	2.016			C_{17} paraffin
13.28	1.796			
13.74	2.352			C_{18} paraffin
14.32	1.555			
14.73	2.456			C_{19} paraffin
15.33	1.209			
15.69	2.430			C_{20} paraffin
16.62	3.697			C_{21} paraffin
17.22	1.750			
17.51	2.350			C_{22} paraffin
17.91	1.130			
18.09	0.813			
18.36	2.598			C_{23} paraffin
18.73	1.269			
19.20	5.860			C_{24} paraffin
20.18	5.328			C_{25} paraffin
21.41	5.528			C_{26} paraffin
22.99	5.320			C_{27} paraffin
25.07	5.163			C_{28} paraffin
27.82	3.534			C_{29} paraffin
31.39	1.360			C_{30} paraffin
35.51	0.219			

Table 5-13.Assay: Plasma Middle Petroleum Residue (μl/l)

Sample Date	Bear Number 1	2	3	4
Oct. 9, 1979				
Oct. 17			0.6	
Oct. 28		0.7		
Oct. 31				
Nov. 2	0.8			
Nov. 13				0
Nov. 16				0
Dec. 10	0	0	0	0
Feb. 15, 1980		0		
Feb. 20		. . . . . [a]		0
Feb. 23		22.8	0	. . . . .
Feb. 26		30.8	. . . . .	35.0
Feb. 29			36.4	23.4
Mar. 6			24.1	
Mar. 11		7.1	1.5	
Mar. 12				4.3
Mar. 15		3.8		
Mar. 20			1.3	
Mar. 21				2.7[b]
Mar. 25			0 [b]	
Mar. 26				
Mar. 29		3.5		
Mar. 30	0			
Apr. 1		2.6		
Apr. 4		5.1		
Apr. 14		16.4		
May 29		2.3		
July 10		0.4		

[a] time of exposure to oil

[b] death of animal

Table 5-14. Residues of Midale crude oil in tissues of Bear 3 and Bear 4.

Tissue	Concentration (μl/kg)	
	Bear 3 (Mar. 25)	Bear 4 (Mar. 21)
Liver	14.6	18.9
Kidney	N.D.[a]	200
Skeletal muscle	N.D.	6.4
Heart muscle	5.0	2.2
Brain	5.7	325
Spleen	2.6	3.2
Subcutaneous fat	N.D.	N.D.
Mesenteric fat	N.D.	N.D.
Marrow	471	3.4
Lung	3.4	9.5

[a] N.D., not detectable

Table 5-15. Residues of Midale crude oil in urine after oil exposure.

Date of Sample	Time of Sample[a]	Concentration (µl/kg)		
		Bear 2	Bear 3	Bear 4
Feb. 22	13.00	154		
Mar. 1	18.00			25
Mar. 7		714	442	
Mar. 9		168		
Mar. 12		157		
	16.00			111
Mar. 13	a.m.	67		
	13.00	137		
Mar. 14	a.m.	98		406
	p.m.	244		
Mar. 15		277		230
	19.00		27	
Mar. 16	a.m.	70		
	p.m.	146		
Mar. 17	a.m.	221		52
	p.m.			36
Mar. 18	a.m.	67		263
	14.30	42		
Mar. 19			71	
Mar. 21	24.00			34
Mar. 22		616	74	
Mar. 23	a.m.	59	91	
Mar. 24		73	65	
Mar. 27		182		

[a] in those cases where time was identified

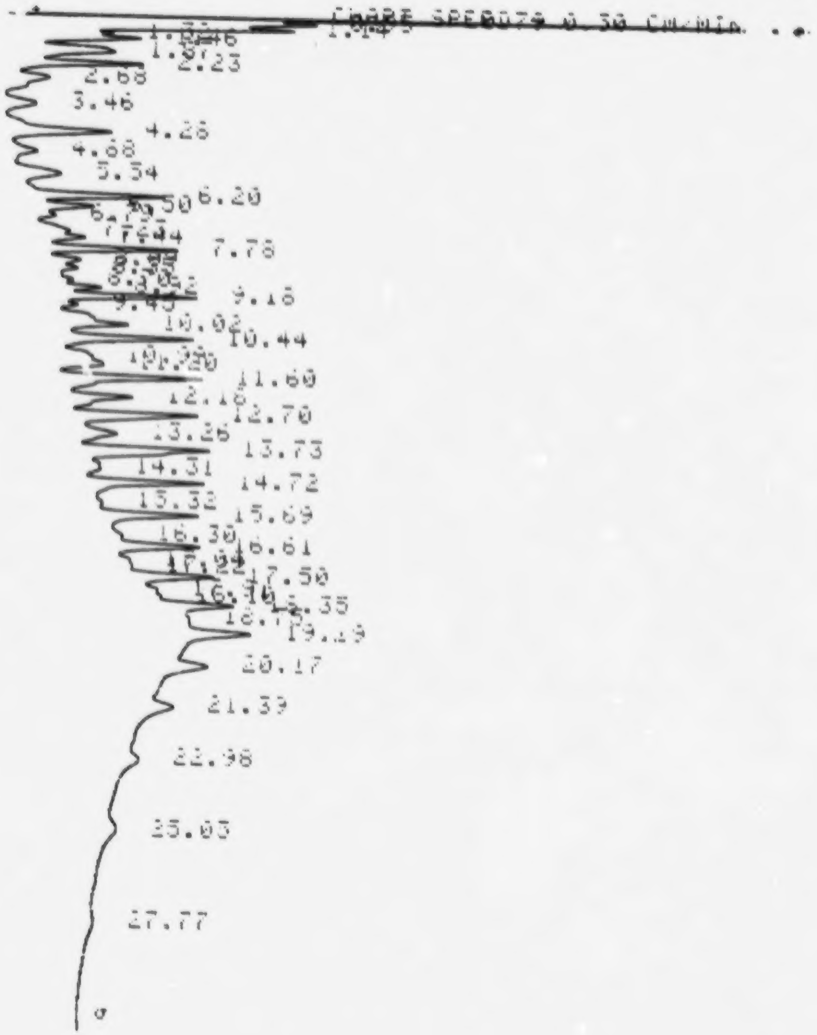

Figure 5-11. Representative gas chromatogram of Midale crude oil residues in feces (Bear 3)

Table 5-16. Quantification of gas chromatogram of feces extract from Bear 2, Feb. 23, 1980.

RT	AREA %	nl or ug/peak	µl or mg/g sample	Compound
0.37	13.070			
0.64	1.575	0.359	9.20	methylbenzene
0.81	0.977			
1.04	0.798			
1.16	0.854	0.240	6.15	dimethylbenzene
1.33	0.316			
1.48	0.724			
1.89	1.093			
2.26	0.901			C_{10} paraffin
2.70	0.689			
2.96	0.413	0.0907	2.33	1,2,3-trimethylbenzene
3.49	0.889			
4.30	1.452			C_{11} paraffin
4.90	0.978	0.169	4.32	1,2,4,5-tetramethylbenzene
5.55	1.027			
6.21	1.203			C_{12} paraffin
6.51	0.722	0.179	4.58	naphthalene
6.80	0.472			
7.24	0.724			
7.45	0.648			
7.79	0.929			C_{13} paraffin
8.10	0.642	0.230	5.91	2-methylnaphthalene
8.37	0.524	0.316	8.10	1-methylnaphthalene
8.71	0.644			
8.93	0.586			
9.19	0.974	0.445	11.4	C_{14} paraffin
9.46	0.485			
10.03	1.599			
10.45	1.396	0.619	15.9	C_{15} paraffin
11.00	0.811			
11.21	0.880			
11.60	1.640	0.642	16.5	C_{16} paraffin
12.19	1.334			
12.71	1.441			C_{17} paraffin
13.27	1.409			
13.74	1.585			C_{18} paraffin
14.19	0.457			
14.32	0.709			
14.73	1.875			C_{19} paraffin
15.32	0.731			
15.69	1.711			C_{20} paraffin
16.30	0.980			

Table 5-16 (Continued).

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
16.62	1.541			C_{21} paraffin
17.05	0.636			
17.22	0.572			
17.51	1.548			C_{22} paraffin
17.91	0.721			
18.10	0.582			
18.37	1.677			C_{23} paraffin
18.73	0.821			
19.20	3.827			C_{24} paraffin
20.18	3.881			C_{25} paraffin
21.42	4.395			C_{26} paraffin
23.01	4.895			C_{27} paraffin
25.10	6.240			C_{28} paraffin
27.87	6.913			C_{29} paraffin
31.44	9.665			C_{30} paraffin

Table 5-17. Quantification of gas chromatogram of feces extract from Bear 3, Feb. 23, 1980.

RT	AREA %	nl or ug/peak	& µl or mg/g sample	Compound
0.35	23.895			
0.79	1.372			
0.95	1.142			
1.14	1.200	0.195	5.42	dimethylbenzene
1.32	0.440			
1.46	0.964			
1.87	1.458			
2.23	1.205			C_{10} paraffin
2.68	1.448			
3.46	1.129			
4.28	1.830			C_{11} paraffin
4.88	1.226	01.122	3.40	1,2,4,5-tetramethylbenzene
5.54	1.270			
6.20	1.460			C_{12} paraffin
6.50	0.866	0.124	3.45	naphthalene
6.79	0.558			
7.23	0.847			
7.44	0.736			
7.78	1.077			C_{13} paraffin
8.09	0.733	0.152	4.22	2-methylnaphthalene
8.35	0.584	0.203	5.66	1-methylnaphthalene
8.70	0.724			
8.92	0.639			
9.18	1.091	0.289	8.02	C_{14} paraffin
9.45	0.520			
10.02	1.726			
10.44	1.486	0.381	10.6	C_{15} paraffin
10.99	0.832			
11.20	0.915			
11.60	1.697	0.384	10.7	C_{16} paraffin
12.18	1.353			
12.70	1.493			C_{17} paraffin
13.26	1.423			
13.73	1.637			C_{18} paraffin
14.31	1.108			
14.72	1.696			C_{19} paraffin
15.32	0.768			
15.69	1.714			C_{20} paraffin
16.30	0.943			
16.61	1.548			C_{21} paraffin
17.04	0.605			

Table 5-17 (Continued).

RT	AREA %	nl or ug/peak	µl or mg/g sample	Compound
17.22	0.555			
17.50	1.550			C_{22} paraffin
17.91	0.720			
18.10	0.616			
18.35	1.663			C_{23} paraffin
18.75	0.937			
19.19	4.246			C_{24} paraffin
20.17	3.810			C_{25} paraffin
21.39	4.059			C_{26} paraffin
22.98	3.958			C_{27} paraffin
25.05	4.063			C_{28} paraffin
27.77	2.926			C_{29} paraffin
31.32	1.493			C_{30} paraffin

Table 5-18. Quantification of gas chromatogram of feces extract from Bear 4, Feb. 26, 1980.

RT	AREA %	nl or ug/peak	μl or mg/g sample	Compound
0.35	24.330			
0.77	1.299			
0.93	1.070			
1.11	0.985	0.145	3.37	dimethylbenzene
1.30	0.396			
1.44	0.668			
1.66	0.908			
2.21	0.626			C_{10} paraffin
2.65	0.294			
3.44	0.129			
4.27	0.387			C_{11} paraffin
4.87	0.140	0.0126	0.293	1,2,4,5-tetramethylbenzene
5.53	0.291			
6.19	0.516			C_{12} paraffin
6.49	0.322	0.0417	0.969	naphthalene
6.78	0.160			
7.23	0.280			
7.43	0.300			
7.77	0.518			C_{13} paraffin
8.09	0.298	0.0559	1.30	2-methylnaphthalene
8.35	0.254	0.0801	1.86	1-methylnaphthalene
8.70	0.275			
8.92	0.304			
9.17	0.608	0.145	3.38	C_{14} paraffin
9.45	0.228			
10.01	0.913			
10.43	0.835	0.194	4.50	C_{15} paraffin
10.98	0.444			
11.20	0.487			
11.59	0.994	0.204	4.73	C_{16} paraffin
12.18	0.779			
12.70	0.929			C_{17} paraffin
13.24	0.867			
13.73	1.025			C_{18} paraffin
14.17	0.243			
14.72	1.739			C_{19} paraffin
15.32	0.730			

Table 5-18 (Continued).

RT	AREA %	nl or ug/peak	µl or mg/g sample	Compound
18.34	4.891			C_{23} paraffin
19.19	4.528			C_{24} paraffin
20.17	4.361			C_{25} paraffin
21.40	4.372			C_{26} paraffin
22.99	4.056			C_{27} paraffin
25.07	3.606			C_{28} paraffin
27.79	2.719			C_{29} paraffin
31.31	1.110			C_{30} paraffin

Table 5-19. Residues of Midale crude oil in feces and bile after oil exposure.

Date of	Concentration (ml/kg or l)				
	Feces			Bile	
Sample	Bear 2	Bear 3	Bear 4	Bear 3	Bear 4
Feb. 22	123				
Feb. 23	893				
Feb. 25			401		
Feb. 26			456		
Feb. 28		595			
Mar. 1			82.6		
Mar. 7	13.4	250	92.2		
Mar. 13		30.7			
Mar. 15		169			
Mar. 16	0.518				
Mar. 17	N.D.[a]		96.0		
Mar. 18	0.288				
Mar. 19		14.4			
Mar. 21					0.963
Mar. 22	230	6.20			
Mar. 23		3.65			
Mar. 25				0.226	
Apr. 4	8.83				

[a] N.D., not detectable

high fecal oil levels. Although this may have been due to extraneous contamination, the chance for this was slight, particularly for the April 4 sample. More probably, oil ingestion occurred through grooming activity since hair was found as a fecal component in all of the analysis.

Bile also contained oil residues as determined by fluorometry. At death, Bears 3 and 4 had 0.226 and 0.963 ml/l of oil in their bile samples.

5.4 Discussion

5.4.1 Characteristics of Midale Crude

5.4.1.1 Physical Characteristics

As seen in Table 5-1, Midale crude oil can be classified as a medium weight crude oil, with a gravity (°API, 60° F) at 27.5 similar to that of Prudhoe Bay crude (Thompson *et al*. 1971, Coleman *et al*. 1973) but less than that of a Kuwait or South Louisiana crude, for example (Pancirov 1974). Crude oils have API Gravities in the range of 10 to 48, corresponding to specific gravities of 0.79 to 1.00 (International Petroleum Encyclopedia 1974, 1975, 1976).

Saybolt Universal Viscosity of Midale crude at 100° F is 86, more viscous than Prudhoe Bay crude at 73.5 or Norman Wells crude at 42 (Charbonnier *et al*. 1969 in Beak Consultants 1974).

5.4.1.2 Chemical Composition

Midale crude has been analyzed to show the presence of appreciable quantities of paraffins and naphthenes (Table 5-3). It is considered as a mixed-base oil for that reason (Bureau of Naval Personnel 1965). Although the compositional analysis by the Shell Refinery Laboratory (Table 5-2 and 5-3) is incomplete, similarities to the Prudhoe Bay crude are again evident in the paraffin composition. In comparison to the reports by Thompson, Coleman, and colleagues on the Prudhoe Bay crude, Midale crude has a smaller percentage of aromatics (about one-half that of Prudhoe Bay) but a larger proportion of naphthenes. The larger proportion of naphthenes, or asphalts, may account for the greater viscosity of the Midale crude.

The gas chromatographic assay carried out in this study verifies the presence of many fractions of low boiling point, including benzenes and paraffins. The large molecular weight n-paraffins are well represented, as are naphthalenic compounds. The chromatogram of Figure 5-2 correlates well with regard to the presence of identifiable fractions with that of Figure 5-1, an older gas chromatographic assay of Midale crude. This suggests that although compositional changes in the oil at the wellhead would undoubtedly have occurred over time (Clark and Brown 1977), these were not so major as to altogether change the characteristics of Midale crude. The past data records are then generally representative of the Midale crude used experimentally in this study. As a point of comparison, a Norman Wells crude chromatogram shows a greater number and intensity of peaks with short retention times (Engelhardt 1978). This greater emphasis on low boiling point fractions in Norman Wells crude is in keeping with its lower viscosity.

Quantification of the chromatogram (Table 5-4) allows comparison of a number of specific standardized fractions with values presented for Prudhoe Bay (Thompson et al. 1971, Coleman et al. 1973), South Louisiana, and Kuwait crudes (Pancirov 1974). The following is a respective listing of the proportion (wt%) of two compound groups of interest in the three latter oils: benzenes - 7.0, 3.9, 4.8; naphthalenes - 9.9, 1.3, 0.7. Midale crude more nearly approaches the composition of Prudhoe Bay crude than that of the other two crudes.

5.4.1.3 Spectral Characteristics

Spectrofluorescence characteristics of Midale crude show general similarities to those of other crude oils determined by conventional fluorescence spectrocopy (Engelhardt 1978, Frank 1975, Wakeham 1976). Particularly relevant to this study are the findings reported by Frank (1975, 1978) that weathering effects were of no significant effect on the fluorescence characteristics. This included the effects of photo-decomposition, as well as air- and water-exposure weathering. In this study as well, the 24-hour and 48-hour weathering had no significant effect on fluorescence. Thus, the concentrations of oil reported in biological samples, as calculated from Midale crude standards, is a real measure of total petroleum concentration in the samples.

5.4.1.4 Weathering of Oil

The concern about weathering effects on the composition of petroleum was prompted by findings (Engelhardt 1978) that Norman Wells crude showed significant changes during 24 hours of exposure on sea water at ambient temperatures in the Arctic (about 4°C). Approximately 50% of the low boiling fractions were removed within 24 hours. The degree of loss in this study was much smaller, attributable to a less light oil character, and relatively fewer low boiling fractions. The changes recorded, a progressive shift in proportion from hydrocarbon fractions of low molecular weight to those of higher molecular weight, were probably of a lower order in the bear-oiling experiment, in that temperatures were lower in the Churchill laboratory - air - 15°C, water 0°C. These lower temperatures would tend to slow down evaporation and dissolution losses. Although weathering effects were then of little significance in this study, they must be taken into consideration in any field spill, in which more time, water movement, and other weathering effects come into play.

Weathering in the arctic environment involves dissolution in sea water and evaporation to the air. The low molecular weight compounds are most affected by this process. As the oil becomes progressively more viscous, evaporation and dissolution will be slowed (Clark and MacLeod 1977), unless wave action is present, under which circumstances the evaporation or loss rate remains high (Smith and MacIntyre 1971). These authors determined that virtually all hydrocarbons smaller than C_{15} will volatilize from the sea surface within about 10 days, the absolute time being dependent on temperature. Hydrocarbons from C_{15} to C_{25} will be mainly retained in the oil slick, and greater than C_{25} will not be lost. Dissolution into the water column can include the formation of macroparticles, emulsions, and dissolved fractions, all of which are also dependent on physical factors such as temperature, wind action, and current velocity. In general, the smaller molecular weight compounds are exponentially more soluble in water than the larger compounds (Clark and MacLeod 1977). For instance, benzene has a solubility of 1,780 ppm, versus naphthalene at 31.3 ppm. Solubility in sea water is less than in fresh water (Eganhouse et al. 1976, McAuliffe 1966). Another weathering factor of immense scope is that of the effect of microbial activity, which can have effects on petroleum composition and quantity in the water column, including in arctic conditions (Karrick 1977).

5.4.2 Accumulation and Clearance

5.4.2.1 Evidence of Exposure

While the physical appearance of the vomitus from all three oiled bears led to the supposition that it contained a high proportion of oil, it was determined that over half of the weight of vomitus was petroleum (Table 5-11). Compositional changes also occurred, with chromatographic evidence (Figure 5-9) pointing to a shift towards the larger molecular weight hydrocarbons. One is that they volatilized to a greater degree from the vomitus because of its higher temperature. This is likely to be of small significance, however, in that the vomitus was very fluid and thin and would have cooled rapidly at laboratory temperatures on the collecting plate. The second possibility, a more likely route of loss, is that these fractions were absorbed preferentially in the stomach immediately after the oil ingestion. The bears did show signs of toxic neurological involvement at this time, attributable to the more acutely toxic low molecular weight fractions.

The presence of oil in the stomach contents of the bears (Table 5-11) more than a month after the oiling event indicates their effectiveness at grooming out even the low basal hair layers of oil which still matted the fur. The conclusion is that the bears must have seen a constant, although perhaps decreasing, exposure throughout the experimental period. This exposure was not of a full range hydrocarbon fraction complex, however, but only of the upper molecular weight range of those fractions in Midale crude. The limited chromatogram of fur extract (Figure 5-10, Table 5-12) supports this conclusion. It would seem that the matting of the fur was so solid that regular grooming would not remove the oil layer unless the hair was pulled away as well. There was always some hair in samples of feces and stomach contents after the oil exposure.

Conclusive proof of the uptake of a contaminant is its appearance in the circulation of an exposed animal. The high levels of oil in plasma in the first week indicate maximum dosing at this time (Table 5-13). The lower concentrations in the later experimental period could be from continued uptake at a lower dose level, as by grooming of the contaminated fur, or evidence of oil in transit from tissue reservoirs to excretory organs. Both conditions are possible in these bears. A rise in Bear 2 plasma oil levels in mid-April is of interest in that it shows that the bear was re-exposed at that time. A probable cause for this were the efforts made at the Assiniboine Zoo to clean residual oil from the inner fur. The effect of the cleaning was probably to lift or otherwise make available some of the oil for grooming and ingestion. The time base of clearance of this input cannot be determined since the next available blood sample was six weeks later.

The plasma levels in the bears may be compared to those found in the oil ingestion studies by Engelhardt et al. (1977) and Engelhardt (1978) on ringed seals. There, controlled doses of Norman Wells crude oil at a rate of 5 ml/day for four to five days resulted in rapid elevations of petroleum-derived hydrocarbons such as benzene and naphthalene. Plasma levels of these hydrocarbons, as well as of petroleum in general, declined within two days of cessation of dosing, more rapidly than what was observed in the dosed polar bears. This points to either a massive dose in the bears, or continued exposure, or a defective clearance mechanism. The two former suggestions are probable, the latter possible. In the seals, plasma did serve as a transit route for excretion of metabolized oil, as water-soluble fractions. No such assessment was possible in this bear study.

Plasma or blood was identified to show accumulated levels of higher molecular weight hydrocarbons, as well. In the codfish (Gadus morrhua), plasma and blood had low but fairly constant levels of benzo(a)pyrene and hexadecane for three days after a single dietary dose (Corner et al. 1976). In ducks also, blood accumulated aromatics after 14 daily doses of Louisiana crude oil at concentrations lower than tissues, particularly adipose (Lawler et al. 1978).

5.4.2.2 Tissue Distribution of Petroleum

Although the distribution pattern of oil residues in the tissues of Bears 3 and 4 was not uniform, the tissues identified as carrying the highest oil load at the time of death were kidney, brain, and bone marrow. This was followed at much lower levels by liver, lung, muscle and spleen, in that order. Residues were found in such tissues also after oil ingestion and immersion in seals (Engelhardt et al. 1977, 1978). The bears differed from the seal study, however, in that no residues were found in adipose tissues in the bears, while seal blubber had appreciable accumulations. This may be a time-related factor of fat utilization. The seal study was of short duration, probably not adequate to create a negative balance as far as storage reserves were concerned. The bears, however, had not fed for approximately five weeks. At such a stage of starvation, fat reserves were utilized and would not have served as an effective storage depot for oil residues.

In an unrelated vertebrate, Neff et al. (1976) found an accumulation of naphthalenes particularly in brain, liver and heart, as well as other tissues, in the killifish, (Fundulus similis). Accumulation in other fish species has been documented by Rice et al. (1977), Hardy et al. (1974), Lee et al. (1972), Anderson et al. (1974), and Roubal and co-workers (1974, 1977). In general, these authors showed that petroleum hydrocarbons exhibit a tissue specificity, concentrating to a greater degree in tissues of high fat content, such as brain or adipose tissue, and in tissues of presumed oil metabolism, such as liver. Not only do the tissues differ in their total hydrocarbon load, but they also are specific to the type of hydrocarbon. The n-alkanes, for instance, tend to accumulate in lipid deposits or fatty tissues in herring, (Clupea harengus) (Whittle et al. 1977) and in salmonids (Roubal 1974). Aromatic hydrocarbons may accumulate more in liver tissues, and the large aromatics to a greater degree than smaller molecular weights (Roubal et al. 1977). A preferential uptake of n-alkanes has also been demonstrated in the cod, (Gadus morrhua) (Hardy et al. 1974).

No test of preferential fraction uptake was carried out in the bears, but may be postulated to have occurred since marked residues remained in tissues several weeks after exposure, when the smaller molecular weight hydrocarbons would have been cleared because of their greater solubility characteristics.

5.4.2.3 Clearance of Petroleum

Urine was demonstrated to be a major route of excretion of Norman Wells petroleum hydrocarbons in the ringed seal studies. After oil immersion for 24 hours, in which ingestion was confirmed to have not occurred, seals had 30 to 40 ul/l oil in urine. Although these concentrations were lower than what was observed in the bears after oiling (Table 5-15), they are in the range of the urine levels of the bears. An overwhelming proportion of urine hydrocarbons in seals was in the form of water-soluble metabolites. In the bears, as well, urinary loss of the hydrocarbon load must be considered significant. Further, this continues as a route of release for the duration of the monitored period.

It is necessary to recall here that the method of solvent extraction used to separate petroleum hydrocarbons from urine would have recovered only the organically-soluble hydrocarbons. Any metabolites, whether in urine or other samples, were not extracted, nor measured.

The predominant route of hydrocarbon clearance post-ingestion was by way of the feces (Figure 5-11). High fecal hydrocarbon levels after oral dosing have been identified in cod (Corner et al. 1976), and in salmon fry, (Oncorhynchus gorbuscha) (Rice et al. 1977). In the dosed bears, some compositional change occurred in feces, as compared to crude oil, suggesting a major uptake of the lowest molecular weight fractions (Tables 5-16, 5-17, and 5-18). The potential for large quantities of oil being absorbed is high, since the fecal samples contained as much as 90% oil right after dosing. Fecal samples taken in the later experimental period still contained oil (Table 5-19). This may have originated from continued ingestion of the oil or else is an artefact of oil in bile.

Bile was found to show the presence of oil in the bears and may thus have contributed to the fecal load (Table 5-19). In this way bile serves as an eventual oil-voiding mechanism, even if allowances are made for reabsorption of a portion of this biliary oil. Bile has been identified as a major route of excretion, particularly for polynuclear aromatics. This has been shown for naphthalene in the killifish after oral dosing (Dixit and Anderson 1977, Neff et al. 1976), as well as in the cod for benzo(a)pyrene and hexadecane (Corner et al. 1976). Engelhardt et al. (1977) found that bile showed high levels of Norman Wells crude oil hydrocarbons even one week after exposure and concluded this to be a major clearance route. Engelhardt (1978) did not find, however, that naphthalene was preferentially cleared by way of bile function in the ringed seal study. Presumably pulmonary loss may have played a greater role there.

6. HEMATOLOGICAL AND BIOCHEMICAL TOXICOLOGY

F.R. Engelhardt and F.A. Juck

6.1 Introduction

6.1.1. General Considerations

This section of the report deals primarily with oil toxicity effects in the dosed polar bears as assessed at the blood level. Both F.R. Engelhardt and F.A. Juck were involved in obtaining the following information; the former author for the purposes of assessing petroleum effects in the experimental period, as related to reference values from bears before oiling or not oil-exposed, the latter for purposes of clinical monitoring of the oiled bears when they became distressed. Similar results were found by the two authors for samples analyzed, each using different methodologies.

6.1.2 Petroleum as a Toxicant

The toxic effects of petroleums on animal and plant organisms have of course been long recognized, and much research has been directed in recent years to identify the nature of the toxicity. Acute and latent or chronic toxicities are recognized, and few, if any, organisms are immune to toxic effects. Several recent review articles and volumes survey the available information in a detail not possible in this report (Smith 1970, Nelson-Smith 1970, Malins 1977, McIntyre and Whittle 1977, Neff 1979, among others). Much of the toxicity of petroleums has been attributed to the aromatic component (Boylan and Tripp 1971, Moore et al. 1973, Moore and Dwyer 1974, Anderson et al. 1974b). Not only the aromatics, however, can be considered as toxic. Certainly low molecular weight paraffins have recognized toxic action, but less is known about these compounds as regards their toxic role in petroleum oils.

Petroleum, such as crude oil, has a common effect across a wide number of species because of its basic mode of action. Work reported by Roubal (1974) and Roubal and Collier (1975), in which they used spin-labelling techniques, discusses the mode of action of petroleum hydrocarbons. In their studies on coho salmon, they found that petroleum hydrocarbons were bound primarily to membranes in neural tissues. Paraffins isolated differently from the aromatics. Paraffins penetrated into the inside of the cell membrane where they associated with its hydrophobic interior face. Aromatic hydrocarbons were bound to various non-polar and electron-interactive sites on the cell membrane surface, there causing perturbations in membrane surface organization. According to membrane theory, such a disturbance will have physiologically significant consequences. Ionic fluxes and osmotic balance will be disturbed, as will electrochemical potential gradients. The latter change will alter neural and muscular reactivities. Further, structural alterations of the membrane of the cell, as well as that of cell organelles such as mitochondria, may change the activities of enzymes in cellular metabolism which may be further modified by structural alterations of receptor sites for regulatory hormones.

Although there is enough evidence to suggest that all species are responsive to petroleum hydrocarbons to some degree, the research effort has certainly not been uniform over the range of species. Most of the work has concentrated on invertebrates, as well as fish, as a survey of the literature readily shows. Mammal species have been little examined as to the effects of this hydrocarbon complex. Additionally, most of the studies have concentrated on the more controversial marine environment. The mammals in this environment are of course less readily obtainable or available for studies of oil toxicity. The only conclusive work on marine mammals has been that by Geraci, Smith and Engelhardt on the ringed seal referred to previously. No definition of toxicity effects is available for the polar bear, even though this species is likely to be under great impact from an oil spill in its environment (Birchard _et al_. 1978). Relatively little comprehensive information is available with terrestrial mammals and other terrestrial species. This is perhaps surprising since it has been estimated that a large portion of the entry of petroleum hydrocarbons into the environment is non-marine, from land-based discharges (National Academy of Sciences 1975).

6.1.3 Fractional Toxicities

The complex nature of petroleum, in particular crude oils, excludes the possibility of completely resolving all specific fractional toxicities. Even the selection of major or readily identifiable fractions and assessing these for toxicity may not compare to their effects in the oil, since synergistic or antagonistic effects are possible and likely probable. It is, however, one of the necessary steps in interpreting petroleum toxicities. This may then lead to some predictive conclusions which will allow first order toxicity assessments of different petroleums based on their compositional characteristics. A small number of petroleum hydrocarbons will be introduced here in consideration of their toxicity effects. The choice of these compounds is predicated on their standarized presence in the Midale crude oil used in this study, as well as on the availability of adequate toxicity information.

6.1.4 Toxicity Tests

6.1.4.1 Blood Disorders

Benzene poisoning produces a complex series of alterations in hematopoietic function and hemoglobin synthesis. The generalized sequence of effects seems to be an initial stimulation of the hematopoietic system under chronic action, terminating with suppression with advanced poisoning. Imperfect, immature erythrocytes are released from the bone marrow which are destroyed earlier than usual (Villani _et al_. 1960). Benzene appears to act in the rabbit by an inhibition of heme synthesis at the stage of inclusion of ferrous iron into the protoporphyrin molecule, at the level of heme synthetase (Kakhna and Muzyka 1970, Myazaki 1960a,b). In the toxic hemopathy produced by benzene, there may be the appearance of the fetal (F) type of hemoglobin, otherwise virtually absent in adults. Up to six times normal values have been found (Aksoy and Secer 1964, Aksoy _et al_. 1972). An increased proportion of Hb-F in an animal will have negative consequences on its oxygen metabolism because of a recognized decreased O_2 carrying capacity of this hemoglobin. This is significant in addition to the other blood insufficiencies. Further evidence of hemoglobin abnormalities comes from the appearance of Heinz bodies in erythrocytes. These are formed from damaged denaturated hemoglobin, as after naphthalene action, shown in the dog and in man (Zeulzer and Apt 1949).

Not only the hematopoietic arena may be affected by petroleum hydrocarbons. Blood cells in circulation may also be damaged. Damage at this level is not repairable by the individual erythrocyte since it lacks the biochemical mechanism for repair functions. Peripheral hemolysis may actually be primed by defective hematopoiesis induced by the same or similar toxicant. This secondary effect results in a release of less viable cells into circulation. Hemoglobin released into the plasma by peripheral hemolysis is subject to increased destruction of its heme portion by breakdown into bile pigments including bilirubin (Truhaut and Bohnon 1963, Perelli et al. 1970). Naphthalene is identified as having hemolytic action, along with a wide array of other contaminants (Fegler 1952, Pernis and Magestretti 1960).

Leukocytic abnormalities have also been atributed to the toxic action of benzene in the bone marrow. The myelotoxicity of benzene is due to a failure in the production of precursor white blood cells, evidenced by aberrations in values for leukocytic nonspecific oxidases and alkaline phosphatase. Anomalous behaviour at the level of leukocyte alkaline phosphatase can also be induced by toluene, xylene, and other aromatic compounds (Pawelski et al. 1964, 1965; Girard et al. 1970; Friborska 1973).

Blood changes as suggested by the above effects can be assessed at a primary level by study of the circulating elements of the blood, that is, by routine hematology.

6.1.4.2 Electrolyte Imbalance

As a consequence of the membrane effects of particularly aromatic hydrocarbons, one may anticipate direct electrolyte shifts in extracellular fluids, including plasma. Major shifts in this physiological bathing medium will alter neural and muscle functioning. Electrolyte shifts may also be a direct consequence of renal damage, or an endocrine lesion shifting the regulatory balance of mineralocortoids from the adrenal cortex.

The main electrolytes under consideration of toxic effects are $Na^+_=$, K^+, Mg^{++}, Cl^-, and HCO_3^- Calcium ion and $PO_4^{\equiv}$ balance has to be considered as well because of their involvement in the mineralization of hard tissue. Anticipated shifts may be as follows: as a consequence of nephrotoxic challenge, characterized by reabsorptive defects, results in the loss of alkali cations, such as Na+, and $PO_4^{\equiv}$ ion, followed by dehydration; adrenocortical insufficiency leading to an abnormal loss of water, Na^+, and Cl^-, and an increased retention of K^+. Multiple factor effects may also come into play, such as hemolysis and renal failure.

Petroleum hydrocarbons have been shown to affect ion balance. Gasoline mixtures, given to rabbits and guinea pigs by inhalation, had the effect of an outflow of K^+ from the erythrocyte with a resultant imbalance of monovalent cations in the plasma and increased urinary loss of both K^+ and Na^+ (Samedov 1966, Kerimov 1966). Naphthalene has been identified as causing inhibition of Na^+/K^+ ATPase activity necessary for cation transport. Potassium depletion in ocular tissues developed along with cataract development in naphthalene poisoned animals (Ikemoto et al. 1969), and may be a causative factor in cataract development by naphthalene. Aromatic hydrocarbons may act similarly on renal tubule ATPases to affect their reabsorption functions. Benzene has been demonstrated to decrease ATPase and other enzyme levels in renal tissues in mice, following repeated sub-acute exposures (Kaminski et al. 1970).

6.1.4.3 Serum Enzymes

Identification of changes in the levels or activities of enzymes in serum has been a routine tool for the identification of specific tissue lesions. It is not specific to the causative agent of the lesion but finds ready application in toxicity testing. It is also useful as an assessment of recovery from a toxic insult, and forms a routine component for screening of toxic damage. Most of the emphasis in this screening is on liver enzymes, since many of the environmental contaminants, including certain hydrocarbons are hepatotoxic. Since organs vary greatly in their enzyme make-up, alterations in organ specific enzymes in plasma or serum can be diagnostic of lytic or biochemical damage. A number of enzymes indicative of hepatic damage have been used in this study. Glutamic oxalacetic transaminase (GOT) is an enzyme diagnostic of liver necrosis as in chemical poisoning (Wroblewski and LaDue 1955), although it is also indicative of myocardial infarction. Red blood cells also usually contain GOT and thus massive hemolysis may cause elevations. Glutamic pyruvic transaminase (GPT) also rises in liver necrosis conditions (Zontine 1961), but shows little change with cardiac damage (Wroblewski and LaDue 1956) and this serves as a check on GOT. Alanine aminotransferase (ALT) will show elevations in acute liver and renal damage (Routh 1970). Serum alkaline phosphatase has been used routinely to screen for certain types of liver damage, although not specific for toxic necrosis (Faulkner et al. 1968, p. 151). Additionally, lactate dehydrogenase (LDH)changes may be indicative of liver disease, although it would require identification of elevation of the liver specific LDH isozyme pattern to identify liver damage definitively (Faulkner et al. 1968, p. 302).

Benzene, although not a particularly potent hepatotoxic agent, has been shown to cause liver insufficiency (Rozera 1960), but a serum enzyme rise is an inconsistent phenomenon, varying with exposure patterns and species (Di Blasi et al. 1964). In chronic human benzolism, the liver-specific LDH isozyme (s-LDH) is usually increased (Lob 1963), but alkaline phosphatase and GPT activities are often decreased (Hanke 1964).

6.1.4.4 Biochemical Function Tests

These tests involve indices of liver function, protein metabolism, as well as liquid and carbohydrate metabolism, identified by changes in plasma chemical components. An increase in bilirubin, or hyperbilirubinemia, may result from prolonged retention of bilirubin or is the expression of hemolysis (Faulkner et al. 1968). It is also useful for early diagnosis of hepatotoxicity. Bilirubin is differentiated into a conjugated (direct) and free (indirect) form, the former of which is indicative of hepatic involvement while the latter is more specific to hemolytic conditions.

Changes in the cholesterol content of plasma have been associated with chemical poisonings including petroleum hydrocarbons. Hypercholesterolemia was identified following experimental inhalation of gasoline vapors in test animals (Abdullaev et al. 1971). Poisoning with liquid paraffins of C_{16} to C_{21} n-alkanes caused a similar effect (Pokrovskii et al. 1978) as did occupational exposure to gasoline in humans (Mekhtiev 1966). This suggests that the measure of cholesterol may be a valid assay for toxic effects of petroleum hydrocarbons in this study.

6.1.4.5 Nitrogenous End-Products

A build-up of nitrogenous products which would normally be excreted in blood is indicative either of a massive protein breakdown, or renal failure. Since renal damage is a potential with hydrocarbon intoxication, changes may be expected in urea nitrogen, creatinine, and perhaps uric acid levels (Faulkner et al. 1968). Increased blood urea nitrogen is associated with acute and chronic nephritis, as well as other kidney or water balance related diseases (Hepler 1958). High serum creatinine levels are found in conditions of nephritis and renal obstruction, and reflect the degree of impairment (Hepler 1958). Uric acid, as a breakdown product from nucleic acid catabolism, may show elevations as a result of hemolysis involving leukocyte breakdown, or also in renal insufficiency, among other miscellaneous conditions (Faulkner et al. 1968).

6.1.5 Hormonal Changes

No attempt can be made in the scope of this study to define the effects of petroleum hydrocarbons on endocrine balance in polar bears. Two hormones or hormone groups were considered, however, as a valuable addition to this study - cortisol and thyroid hormones (triiodothyronine, T_3, and thyroxine, T_4).

Cortisol has a significance as a stress-responsive steroid, with a multitude of regulatory actions including water balance and calorigenesis. Thyroid hormones were also considered, mainly because of their calorigenic potential and because in other studies on seals there was an inverse relationship between cortisol and the thyroid hormones after oil dosing (Engelhardt, in press). In view of their calorigenic activity, a balance has relevance to the metabolic measures presented by Øritsland _et al_. in another section.

Adrenal hypersecretion of cortisol is associated with absorption of toxic quantities of benzene (Iannaccone and Cicchella 1959, Kanner 1964). In chronic exposures, however, there may be an eventual decline of cortisol levels in plasma because of an exhaustion of the adrenal cortex. Chronic exposure of humans to benzene or toluene produces an adrenocortical dysfunction leading to an inadequacy of steroid synthesis (Kanner 1963, Di Blasi 1965, Makotchenko 1971). Treatment of animals with xylene had a similar effect (Kashkin _et al_. 1968). In the xylene exposure the progression is from an immediate hyperadrenocorticalism, which later reverses to a hypoadrenal state due to exhaustion of functional capacity by continuous toxic action.

Thyroid stimulation has been recorded following exposure to benzene, along with an adrenocortical stimulation. The suggestion is made that benzene acts by enhancing generally the secretion of anterior pituitary hormones (Iannaccone and Cicchella 1959). Any thyroid change of significant duration should affect calorigenesis and heat balance of the bears.

6.1.6 Relevance of Assays

Although the rationale for examining the two hormone groups would appear to be clear, it is perhaps less so when the apparent overlap of tests for liver function and kidney activity are concerned. The problem confronting the researcher is simply inadequate available information with regard to polar bear clinical parameters. Even more germane is the lack of knowledge about response vectors of these parameters. As an example, whereas the clinical enzymes are reputedly diagnostic of liver damage, some of them may not be in exotic species. A liver specificity would have to be determined. St. Aubin and Geraci (1977) and Engelhardt (1979) found that diagnostic enzymes assessed in phocid seals do not always have the same tissue distribution or response specificity as presumed by routine clinical interpretations. Thus, it is necessary to examine several of the standard enzymes for hepatotoxicity to establish a trend of response which will be diagnostic.

6.2. Materials and Methods

6.2.1 Sampling Procedure

Blood was taken by Vacutainer assembly (Becton, Dickinson & Co., Ltd., Mississauga, Ontario) from a femoral artery or vein following immobilization of the bears. At capture and transport, immobilization was carried out by intramuscular injections of Sernylan (phencyclidine hydrochloride, Bio-Ceutic Laboratories Inc., St. Joseph, Missouri) and Sparine (promazine hydrochloride, J. Wyeth and Brother, Ltd., Windsor, Ontario). During handling and blood sampling procedures in the laboratory, immobilization was accomplished by intramuscular injections of a mixture of Ketaset (ketamine hydrochloride, Rogar/STB, BTI PRODUCTS, London, Ontario) and Rompun (xylazine hydrochloride, Baychem Corporation, Kansas, Missouri). An initial dose of 5.5 mg of each drug/kg of body weight was given, followed by additional injections of the drugs in order to prolong immobilization if required. Smaller doses, as low as 3.3 mg/kg body weight, were used on bears in weakened condition.

Once blood samples were obtained, whole blood in potassium-EDTA was cooled and kept for shipment and hematological analysis. Plasma and serum samples were obtained immediately following sampling, and kept frozen for shipment. Transit time for shipment of the samples from Churchill to Ottawa, during which time blood was kept at 4° C and plasma and serum in frozen condition, varied from 2 to 4 days. Hematological analyses were carried out immediately on arrival at Ottawa, while the frozen plasma and serum were stored at -70°C.

6.2.2 Hematological Analysis

Blood characteristics were assessed in order to determine the effect of oil exposure on the peripheral and hematopoietic blood system. All hematological assays were carried out on blood stored in potassium-EDTA. Standard and routine techniques were used in all instances, such as determined by Schalm *et al*. (1975).

Hematological assays carried out by F.R. Engelhardt were assessed as follows. Erythrocytic parameters included determination of hematocrit (packed cell volume - PCV), red blood cell count, and hemoglobin concentration. The hematocrit was determined by the microhematocrit method. Red blood cell counts were done manually using hemacytometers (A-O Spencer Bright-Line), following dilution of an aliquot of the sample using Unopettes (Becton-Dickinson, Test 5851). Hemoglobin concentration was assessed by the cyanmethemoglobin test, using Hycel methods and reagents (Hycel Inc., Houston, Texas). The erythrocytic indices, mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC), were calculated from the above three parameters. All erythrocytic and other hematological assays were carried out in triplicate.

With regard to the leukocytic assays, total white blood cell counts were also carried out manually by hemacytometry and using Unopettes (Becton-Dickinson, Test 5805). Relative white blood cell differentials were calculated from blood smears stained with Giemsa stain.

Routine hematology data obtained by F.A. Juck used the following methods: White cell count and red cell count - Coulter Counter Model ZBl - diluent Coulter Isoton. Hemoglobin - Coulter Hemoglobinometer - diluent Coulter Isoton. Packed cell volume - IEC Micro Capillary Centrifuge, Model MB. Blood differential smears - Ames Hema-Tek Stain Pak - modified polychrome methylene blue.

6.2.3 Blood Biochemistry

6.2.3.1 Overview

Plasma or serum was analyzed for a spectrum of chemical components which would be diagnostic of illness caused by oil exposure, or at least indicate target sites of action of the toxicant. All chemical assays except for hormone determinations were carried out on serum.

Electrolyte concentrations were assessed. Changes in levels of sodium, potassium, calcium, and phosphate measured as Na^+, K^+, Ca^{++} and inorganic phosphate, may be indicative of dysfunctions in salt and water balance, possibly originating at the level of the kidneys, of endocrine origin, or caused by shifts in fluid balance resulting from, for example, diarrhea or polyuria. Determination of serum total protein aided in this assessment.

Elevations in serum levels of diagnostic enzymes may be associated with lesions at a tissue or organ level. These may be biochemical in nature, or extend to a frank destruction of the tissue. A number of enzymes were assessed, particularly glutamic oxalacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), alkaline phosphatase, lactate dehydrogenase (LDH), and alanine amino-transferase (ALT). It was of additional interest to correlate any toxic hemolysis occurring after oil exposure with the capacity of the liver to handle blood pigments originating from red blood cell breakdown. Bilirubin levels were measured for this purpose.

Glucose and cholesterol levels were assessed in serum as general indicators of nutritional states such as fasting periods.

Toxic involvement of the renal excretory system was assessed further by its capacity to handle nitrogenous waste products. Changes in serum levels of nitrogenous end products of protein metabolism, particularly increases in serum urea nitrogen, creatinine, and uric acid, were presumed indicative of renal problems.

The potential effect of oil contamination on endocrine balance was tested by following changes in two hormone categories. Determinations were carried out for plasma cortisol, and for the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4).

6.2.3.2 Clinical assay methods

The following assay methods were employed by F.R. Engelhardt. A large portion of the chemical assays on serum could be carried out by standard clinical chemistry techniques using a Technicon Autoanalyzer instrument (Model SMA 12/60 Micro). The methodology for the specific assays were as described in Technicon technical bulletins, and are of a controlled and standardized nature to allow comparative interpretation of resulting data. For purposes of completeness in this report, the following lists the Technicon methods used:

Assay	Technicon Industrial Method No.
Na+, K+	
Ca++	339-07
Inorganic phosphate	339-03
Total protein	339-04
GOT	339-14
Alkaline phosphatase	339-10
LDH	339-06
Glucose	339-18
Cholesterol	339-19
Uric Acid	339-26
	339-13

A number of other clinical chemistry assays were carred out by manual methods using Sigma clinical assay methods and reagents (Sigma Chemical Co., St. Louis, Missouri). The individual methods may be summarized:

Assay	Sigma Technical Bulletin No.
GPT	
ALT	505
Urea nitrogen	57-UV
Creatinine	640
	555

All assays were carried out in duplicate on serum. The author F.A. Juck employed the following methodologies: Blood urea nitrogen - Worthington #27025 mg % @ 30°) Kinetic method - Talke & Schubert (1965). Creatinine - Worthington #27942 (mg % @ 30°) Colorimetric - Jeffe. Glucose - Worthington #27614 (mg % @ 30°) Kinetic - Bondar & Mead (1974). Total Protein - American Optical Refractometer (gm %). Electrophoresis -

Beckman Microzone System using Barbital Buffer pH 8.2 Ponceau S stain, Cellulose acetate membranes. Bilirubin - colorimetric Jendrassik & Grof., total and direct - diazotization in the presence of caffeine or acid and sodium benzoate - direct bilirubin is determined by diazotization at an acidic pH at which indirect bilirubin is insoluble and nonreacting. SGOT - Worthington #27905 (IU/liter @ 30°), Kinetic - Henry, Amador and Wacher (1962). SGPT - Worthington #27913 (IU/liter @ 30°), Kinetic - Henry et al. (1960). Alk. phosphatase - General Diagnostics #35950 (IU/liter @ 37°) Colorimetric - Phenolphthalein monophos. CPK - Worthington #27251 (IU/liter @ 30°), Kinetic - Oliver & Rosalki. Amylase - Worthington #27681 (IU/liter @ 30°), Kinetic - sol. starch substrate. Lipase - Princeton Biomedix #8210 - 01) Cherry - Crandal Units @ 37°C). Cholesterol - Worthington #27572 (mg % @ 30°), Kinetic - Allain et al. (1974). LDH - Worthington #27602 (IU/liter @ 30°), Kinetic - Wacker et al. (1956). Arginase - Pitman-Moore Chem assay #79116, Colorimetric (IU/liter @ 37°). Calcium - Corning Calcium Analyzer 940 (mgm %). Phosphorus - Worthington 27319 (mgm %), Colorimetric - phosphomolybdate. Magnesium - Bio-Analytics #2920 Colorimetric (mgm %). Sodium and Potassium - Beckman Klina Flame Photometer (mEq/liter). Chloride - Corning Chloride Meter 920 M (mEq/liter). Uric Acid - Worthington #27604, Kinetic (mg % @ 37°). Routine methods were used for evaluation of CSF and urine sample.

6.2.3.3 Plasma hormones

Hormone concentrations were determined by radioimmunoassay on plasma samples as described by Engelhardt and Ferguson (1980). The levels of T_3, T_4, and cortisol were assessed by Immo-Phase methods and reagents (Corning Medical Diagnostics, Medfield, Mass.). In these procedures, an antibody which specifically binds the appropriate hormone was reacted with a trace amount of 125 I-labeled antigen and a test plasma sample. Hormones were released from protein binding by specific releasing agents, 8-anilino-naphthalene sulfonic acid (ANS) in the case of T_3 and cortisol, and thimerosal in the T_4 assay. The amount of labeled antigen bound by the antibody decreased as the concentration of unlabeled antigen in the sample increased, due to competition for antibody binding sites. The antibodies, which are covalently bound to glass particles, were separated from unbound antigen by centrifugation, followed by decanting of the supernatant containing the unbound antigen. The activity of labeled antigen bound to the antibody was assessed by counting the precipitate in a gamma counter. Results were quantified by extrapolation from a standard curve, constructed by substituting standards of known hormone concentration for the sample in the assay.

Oxidative losses and losses caused by repeated thawing of the samples was minimized by storage at -70° C, and completing the assays for all three hormones on the same day for a given plasma sample. All assays were carried out in duplicate on individual samples.

6.3 Results

6.3.1. General Considerations

All of the hematological data and plasma or serum biochemical composition has been compiled for the four bears in tabular form. Table 6-1 describes pre-oiling values for the bears, assumed as control or at best reference values for this study. Tables 6-2 to 6-5 list the hematological and chemical data for the individual bears from capture, on into the experimental period. This includes the recovery phase for Bear 2.

6.3.2 Hematology

6.3.2.1. Erythrocytic Parameters

Prior to the oil exposure events in late February, all four bears showed a stable hematocrit ranging from 45 to 56 % among the bears, while each bear showed only a small variability during the 4 months of laboratory holding. Within one week from the short immersion exposure (Bear 2 - 15 min; Bear 3 - 50 min; Bear 4 - 30 min), the hematocrit showed a small but consistent decline in all dosed bears ranging from 3 to 7% in absolute values. These showed a further, and drastic, decline for the next 5 weeks ending with hematocrits of 14% in Bear 2, and at points of death in Bears 3 and 4 of 28 and 30%, respectively. This represented declines of one-quarter to two-thirds of normal values. Bear 2 was moved to the Assiniboine Zoo at Winnipeg at the end of March and received extensive health care and rehabilitation there. These efforts are described in Appendix 6 to this report. The hematocrit values for Bear 2 increased gradually to an effectively normal value of 49% by July, 1980.

The pattern of change in red cell count and blood hemoglobin concentration paralleled that of the hematocrit changes. The temporal pattern was the same in all bears. Maximum decreases from control values of approximately 6 to 8 million erythrocytes/μl occurred. Bear 2 showed a drop to one-quarter of its normal count by five weeks post-exposure, Bears 3 and 4 to about two-thirds of their normal values. In the latter two instances, the absolute magnitude of the drop in measured RBC count was less than that of the hematocrit change. Bear 2 recovered to approach normal values by July.

Table 6-1. Hematology and biochemistry data taken pre-oiling from four bears, representing normal reference values (data from F.R. Engelhardt).

PARAMETER	n	AVERAGE	ABSOLUTE*	RANGE
WBC (x10^3/μl)	8	8.71		5.3 - 12.15
Neutrophils (%)	7	63	5.48	40 - 80
Bands (%)	7	8.6	0.74	2 - 13
Eosinophils (%)	7	2.3	0.20	0 - 4
Basophils (%)	7	1.8	0.15	0 - 5
Lymphocytes (%).	7	17	1.48	13 - 27
Monocytes (%)	7	6.3	0.55	0 - 14
RBC (x10^6/μl)	8	6.92		5.95 - 8.31
PCV (%)	8	49.4		45 - 55.7
Hgb (g/dl)	10	17.3		15.6 - 19.5
MCV (fl)	8	71.7		66.2 - 81.0
MCH (pg)	8	25.3		23.4 - 28.0
MCHC (g/dl)	8	35.2		34.0 - 36.9
BUN (mg %)	12	12.2		4.8 - 33.4
Creatinine (mg %)	12	1.38		0.7 - 2.5
Glucose (mg %)	12	190.7		115 - 286
Total protein (gm %)	12	7.0		6.2 - 7.8
Bilirubin - Total (mg %)	12	0.15		0 - 0.8
SGOT (IU/l)	12	62.7		27 - 129
SGPT (IU/l)	12	21.5		10 - 52
Alk. phos. (IU/l)	12	46.6		8 - 105
CPK (IU/l)	-	not available		not available
Lactic dehydrogenase	12	393		284 - 632
Amylase	-	not available		not available
Lipase	-	not available		not available
Cholesterol (mg %)	12	311		232 - 392
Uric acid (mg %)	12	0.8		0.5 - 2.1
Arginase	-	not available		not available
ALT (units/ml)	13	25		9 - 62

Table 6-1 (Continued).

PARAMETER	n	AVERAGE	ABSOLUTE*	RANGE
Calcium (mg %)	12	8.95		8.2 - 10.4
Phosphorous (mg %)	12	4.3		4.3 - 8.3
Magnesium	-	not available		not available
Sodium (m Eq/1)	12	134.5		128 - 141
Potassium (m Eq/1)	12	4.0		3.2 - 4.6
Chloride (m Eq 1)	-	not available		not available
Thyroid (ng/ml) - T3	12	2.4		1.45 - 3.74
- T4	12	23.0		17.2 - 38
Cortisol (ng/ml)	12	138.0		35 - 275

*$(x10^3/\mu l)$

Table 6-2. HEMATOLOGY AND BIOCHEMISTRY BEAR # 1

W - VSB Winnipeg
O - R. Engelhardt

PARAMETER	Nov. 2		Dec. 10		March 30	
	W	O	W	O	W	O
Total Leukocytes (x10^3/μl)		8.25			14.6	
Neutrophils (%)		40			85	
Bands (%)		13			1	
Eosinophils (%)		3				
Basophils (%)		3				
Lymphocytes (%)		27			11	
Momocytes (%)		14			3	
Metamyelocytes (%)						
Myelocytes (%)						
Other						
RBC (x10^6/μl)		6.88			4.76	
PCV (%)		48.5			36	
Hemoglobin (g/dl)		16.7		17.0	11.5	
MCV (fl)		70.5			75.6	
MCH (pg)		24.2			24.2	
MCHC (g/dl)		34.4			31.9	
Metarubricytes						
Other						
BUN (mg %)		6.7		6.7	12.1	10.3
Creatinine (mg %)		.7		1.0	.75	.6
Glucose (mg %)		206		188	123	147
Total Protein (gm %)					8.1	
Albumin (gm %)					4.04	
α_1 globulin (gm %)					.43	
α_2 globulin (gm %)					.74	
β_2 globulin (gm %)					1.72	
γ globulin (gm %)					1.17	
A/G ratio					1.0	

Table 6-2 (Continued).

PARAMETER	Nov. 2		Dec. 10		March 30	
	W	O	W	O	W	O
Bilirubin-						
Total (mg %)		0.2		0.0	0.3	0.2
Direct (mg %)					0.1	
Indirect (mg %)					0.2	
SGOT (units)		50		42	25	24
SGPT (units)		10.0		10.0	10	12.0
Alk. phos. (units)		16.0		25.0	28.7	51.0
CPK (units)						
Amylase (units)					94	
Lipase (units)					.76	
Cholesterol (mg %)		325		308	260	347
Uric acid (mg %)		0.9		0.8		0.6
LDH (units)		336		382		544
Arginase (units)					0.0	
ALT (units)		13		11		10
Calcium (mg %)		9.1		9.1	9.35	9.5
Phosphorus (mg %)		5.7		5.8	6.91	7.5
Magnesium (mg %)					1.91	
Sodium (m Eq/l)		138		134	136	143
Potassium (m Eq/l)		3.6		3.8	4.7	3.8
Chloride (m Eq/l)					99	
Thyroid hormones (ng/ml)						
T3		2.51		3.0		1.94
T4		38		29		53
Cortisol (ng/ml)		218		29		259

Changes in blood hemoglobin paralleled hematocrit changes in all bears almost exactly, both in temporal pattern and magnitude. The range of control hemoglobin concentrations in the four bears was from 16 to 20 g/dl, each individual bear showing about a 0.5 to 2 g/dl variability in the predosing period.

The mathematically calculated erythrocytic indices, MVC, MCH and MCHC, evidence the absolute changes in the cellular assays above. The MCV values showed no significant early change after oiling from control values of 66-81 fl and then exhibited a small order decline in all three oiled bears by the fifth week following exposure. A rebound appears to have occurred in Bear 2, suggestive of a hematopoietic recovery, starting at the end of March, and continuing during its therapy into April, achieving normal MCV values by July.

Control concentrations for MCH were of the order of 23 to 28 pg per erythrocyte. In the cases of Bears 2 and 3, the post-oiling changes paralleled those of the MCV alterations, both temporally and quantitatively, while Bear 4 was not found to show any significant changes in MCH during its trial period.

Values for MCHC, normal at 34 to 37 g/dl, were also found to not change within one week after the oil exposure. Thereafter, the trend was for a small order decline, followed by a small rise above control values by the fifth week after exposure. In the case of Bear 2, MCHC values varied irregularly during the recovery period, ending with control levels by July.

A summary of the erythrocytic effects observed during the period after oil exposure in the Churchill laboratory is presented in Table 6-6. It can be readily seen that effects on red blood cell parameters were of low magnitude during the first week following exposure. The hematological effects appear to be more latent, being generally maximum in their negative effects by the fifth week following exposure. Only in the case of MCHC was some rebound noted by this time.

6.3.2.2 Leukocytic Parameters

Whereas the erythrocytic changes were major and consistent, changes in white blood cell characteristics were more variable. Total white blood cell counts were in a range of 5 to 12 thousand leukocytes/μl prior to oil exposure. A rise in white blood cell count occurred in Bear 2 within one week after the exposure, and in the other two exposed bears by the second week. The increases were to levels of 11 to 12 thousand cells/μl. After that point in time, white blood cell changes were inconsistent, showing declines and elevations in all bears by the fifth week. It is perhaps of note that in both Bears 2 and 4, the lowest values of leukocyte count were observed by the end of March, at 4.5 and 4.4 thousand cells/μl, respectively. The recovery period of Bear 2 at Winnipeg was marked by a major increase in the total white blood cell count by mid-April, followed by a decline to normal levels by July.

With regard to leukocyte differentials, only the neutrophils and lymphocytes showed any change although not major in either case. Pre-experimental control value ranges were as follows: segmented neutrophils, 40-80%; immature neutrophils, 2-13%; eosinophils, 0-4%; basophils, 0-5%; lymphocytes, 13-27%; monocytes 0-14%. In the first week after exposure, Bear 2 and Bear 4 showed a small rise in their segmented neutrophil proportion, while in Bear 2 and Bear 3, the lymphocyte percentage was assessed as showing a decline, in absolute percentage approximately equal to the neutrophil increases.

During the two to five week period, the segmented neutrophils in bears 2 and 3 were still higher than normal; although, in Bear 2, samples taken at the end of March and beginning of April showed a decrease in segmented neutrophils. This was followed by a rebound to higher than normal values in Bear 2, ending with a decline into a low normal range by July. There was a response in the small proportion of immature neutrophils during the post-exposure period to the end of March. Bears 2 and 3 both showed a decline in the proportion of this cell type, with the possibility of a similar trend in Bear 4. Bear 2 again exhibited a recovery, in that immature neutrophils increased in proportion during the treatment period in Winnipeg. Lymphocytes showed a generally reciprocal relationship to the changes in segmented neutrophils, observed in the later post-exposure period as well. A marked increase in the proportion of lymphocytes was noted in Bear 2 in the blood samples taken at the end of March and early April.

A summary of leukocytic changes relevant to the post-oiling period at Churchill is presented in Table 6-7. Relative changes as compared to pre-exposure individual control values are indicated.

Table 6-3 HEMATOLOGY AND BIOCHEMISTRY BEAR # 2

W - VSB Winnipeg
O - R. Engelhardt

PARAMETER	Oct. 28		Dec. 10		Feb. 15		Feb. 23		Feb. 26		March 11		March 15		March 26	
							Day 2 PO		Day 5 PO		Day 19 PO		Day 23 PO		Day 34 PO	
	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O
Total Leukocytes ($X10^3 = /\mu l$)				7.9		7.85				12.0	12.3	10.6	8.8		12.5	
Neutrophils (%)						70.0				84	86	90	83		94	
Bands (%)						12				8		1			1	
Eosinophils (%)						4				1		0				
Basophils (%)						0				0		0				
Lymphocytes (%)						14				7	9	9	17		4	
Monocytes (%)											5					
Metamyelocytes (%)																
Myelocytes (%)																
Other															1	
RBC ($x10^6//\mu l$)				8.31		7.49				6.46	5.6	5.33	4.8		4.87	
PCV (%)				55.7		52.5				47.0	40	39.0	35		30	
Hemoglobin (g/dl)				19.5		18.7		18.7		17.0	13.1	13.8	10.7		11.4	
MCV (fl)				67.0		70.1				72.8	72.7	70.4	72.9		61.6	
MCH (pg)				23.4		25.0				26.3	25.7	28	22.3		23.4	
MCHC (g/dl)				35.0		35.6				35.2	36.2	35	30.6		38.0	
Metarubricytes																
Other																
BUN (mg %)		33.4		11.6		14.8		4.5		4.7	11.4	10.3	32.4	28.2	149	
Creatinine (mg %)		2.5		1.3		1.2		0.9		1.4	1.04	1.2		2.6	11.25	
Glucose (mg %)		176		190		224		174		133	120	201		204	162	
Total Protein (gm %)		6.2		7.2		6.3		6.0		5.3	8.5	8.1	9.9	9.2	11.0	
Albumin (gm %)											4.52				6.4	
α_1 — globulin (gm %)											0.75				0.8	
α_2 — globulin (gm %)											0.62				0.7	
β — globulin (gm %)											1.41				1.2	
γ-globulin (gm %)											1.19				1.9	
A/G ratio											1.14				1.39	
Bilirubin-																
Total (mg %)		0.8		0.0		0.0		0.4		0.5	3.8	8.9		10.4	10	
Direct (mg %)											2.5				7.0	
Indirect (mg %)											1.3				3.0	
SGOT (units)		129		39		62		56		157	44	61		37	20.0	
SGPT (units)		46.0		20.0		52		18		118	25	18		22	120	
Alk. phos. (units)		86		27		66		19		258	179	56		56	18	
CPK (units)											73				106	
Amylase (units)											32				49	
Lipase (units)											1.1				1.6	
Cholesterol (mg %)		323		392		357		284		238	556	447		537	600	
Uric acid (mg %)		0.5		0.7		1.1		0.7		0.8		1.0		0.4	2.53	
LDH (units)		294		298		420		556		794	318	832		660		
Arginase (units)											5.0				4.5	
ALT (units)		9		24		18		22		13		10		26		
Calcium (mg %)		8.2		10.4		9.6		8.6		8.6	9.4	8.7		9.0	9.95	
Phosphorus (mg %)		4.3		5.0		4.5		5.2		5.2	5.2	4.6		7.2	6.08	
Magnesium (mg %)											1.81				3.08	
Sodium (m Eq 1)		129		134		138		125		130	132	144		124	126	
Potassium (m Eq/1)		3.6		4.2		4.4		3.7		3.4	3.9	3.2		3.5	3.6	
Chloride (m Eq/1)											94				71	
Thyroid hormones (ng/ml)																
T3		1.98		2.75		3.74		5.41		4.24		3.13		7.52		
T4		38		23		32		43		27		33		43		
Cortisol (ng/ml)		90		275		186		40		49		80		333		

PO - Post oiling
(1). toxic signs and granules in granulocytes.
(2). 1+ poikilocytosis; 1+ macrocytes; 2+ anisocytosis;
1+ polychromasia Howell-Jolly Bodies; Leptocytes and Rouleaux.
(3). Howell-Jolly Bodies.

TABLE 6-3 (Continued)

March 29		April 1		April 4		April 14		May 29		July 10		Sept. 24	
Day 37 PO		Day 40 PO		Day 43 PO		Day 53 PO		Day 98 PO		Day 140 PO		Day 215 PO	
W	O	W	O	W	O	W	O	W	O	W	O	W	O
4.5		9.6		8.1	10.38	20.4	21.00	11.8		7.5		9.8	
50		48		82		85		71		55		67	
				1		4		2		3			
								8		18		6	
50		51		16		9		7		21		22	
		1				2		12		3		5	
		Double bodies				(1)							
2.06		1.81			1.79	3.21	2.28	5.71	5.40	6.55		6.71	
15		14			13.8	19	23.0	42.5	45.0	49		46	
3.5		3.8			4.7	6.1	6.9	14.8	16.7	17.6		16.6	
72.8		77.3			77.1	59.1	100.8	74.4	83.3	74.8		68.5	
17.0		17.0			26.3	19.0	30.3	25.9	30.9	26.9		24.7	
23.3		27.1			34.1	31.1	30.0	28.7	37.1	35.9		27.7	
				1/100wbc		3/100wbc							
						(2)		(3)					
167.5	114.5	127	109.5	95	81.8	36	32.0	13.7	13.5	11.5	7.3	7.06	
10.4	9.6	10.8	11.3	6.6	8.8	2.81	3.1		1.1	1.59	1.7	1.3	
152	181	272	325	128	193	124	146	120	140	128	128	170	
8.0	5.8	7.9	6.0	6.2	5.9	8.7	7.3	9.4	8.4	8.45	7.4		
4.85								3.61		3.33			
0.56								0.52		0.7			
0.56								0.92		0.88			
0.94								1.58		1.87			
1.13								2.78		1.61			
1.54								0.95		0.66			
4.0	1.6	5.4	1.8	2.7	3.0	0.9	0.5	0.0	0.1	0.6	2.0	0.0	
1.7		1.0		2.5									
2.3		4.4		0.2									
33	38	190	258	139	217	26	35	43	31	24	39	20	
12	12	17	13	16	12	12	12	24	21	17	21	11	
27.5	50	39	57	22	38	8.5	16	18	28	23	36	44.2	
108		222		83		22		33		26		36	
4.0		41				236		74		7.0		98	
0.66		0.79				0.64		1.05		1.14		0.72	
550	528	500	539	500	538	385	492	400	374	310	364	248	
	0.4	1.01	0.5	0.92	0.6	1.04	0.7	1.57	1.0	1.37	1.0	1.33	
	468		642		1024		810	176	230	220	326		
0.0				0.25		1.5		2.9		0.0			
	11		20		13		12		33		22		
8.89	8.1	8.74	8.3	10.15	9.7	8.67	8.7	9.49	9.3	8.87	9.0	9.09	
8.31	9.4	4.19	5.0	6.22	6.7	7.04	8.0	5.78	6.8	4.87	6.0	5.85	
2.88		2.85		2.68		2.21		2.26		2.26		2.63	
120	122	119	118	128	128	134	137	137	140	134	137	141	
3.1	3.4	2.8	2.4	2.7	3.6	4.9	3.7	4.8	4.8	4.8	4.6	4.4	
90		81		93		94		100		102		104	
	3.70		3.69		3.32		1.03		2.80		1.70		
	11		29		31		27		43		31		
	215		409		431		384		382		192		

Table 6-4. HEMATOLOGY AND BIOCHEMISTRY BEAR # 3

W - VSB Winnipeg
O - R. Engelhardt

PARAMETER	Oct. 17		Oct. 31		Dec. 10		Feb. 23		Feb. 29		Mar. 06		Mar. 11		Mar. 20		March 25	
									Day 2 PO		Day 8 PO		Day 13 PO		Day 22 PO		Day 27 PO	
	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O
Total Leukocytes (x10^3/μl)				12.15		5.3		11.8		9.6		11.5		7.2	11.3	9.43	9.6	10.73
Neutrophils (%)				52		70		80		83		88		70	95		87	
Bands (%)				12		3		5		5		2		3				
Eosinophils (%)				2		2		3		2		0		0				
Basophils (%)				5		0		0		0		0		0				
Lymphocytes (%)				17		24		13		10		12		27	5		12	
Monocytes (%)				12		0		0		0		0		0			1	
Metamyelocytes (%)																		
Myelocytes (%)																		
Other																		
RBC (x10^6/μl)				7.02		5.95		6.69		6.12		5.75		4.75	4.15	4.53	5.24	4.99
PCV (%)				51.5		48		49		46		42		35	30	30	31	28.5
Hemoglobin (g/dl)				18.3		17.6		18.2		16.1		15		13	10.1	9.9	11	11.2
MCV (fl)				73.4		81.0		73.2		75.1		70.5		64	72.3	66.2	59.2	57.1
MCH (pg)				26.1		28		27.0		26.3		25.0		26	24.3	21.9	20.9	22.4
MCHC (g/dl)				35.5		34		36.9		35.0		36.0		37	29.7	33	28.2	39.3
Metarubricytes																		
Other																		
BUN (mg %)		6.9				4.8		17.2		3.9	11.4	19.8		39	124.0	122.8	272.7	153.8
Creatinine (mg %)		1.4				1.6		1.8		1.4		2.2		3.0	8.19	7.3	13.05	12.8
Glucose (mg %)		115				205		206		234	120	148		154	146	159	157	187
Total Protein (gm %)		7.0				7.3		6.5		9.6	8.5	7.9		8.7	11.4	7.8	13.3	9.6
Albumin (gm %)															6.2		6.6	
α_1 — globulin (gm %)															1.0		1.3	
α_2 — globulin (gm %)															0.9		1.2	
β — globulin (gm %)															1.8		2.6	
γ – globulin (gm %)															1.5		1.6	
A/G ratio															1.19		0.99	
Bilirubin-																		
Total (mg %)		0.2				0.1		0.0		1.8	3.8	4.0		3.5	2.2	1.7	1.6	1.5
Direct (mg %)											2.5				1.6		1.0	
Indirect (mg %)											1.3				0.6		0.6	
SGOT (units)		38				27		85		92	41	58		82	19	17	34	60
SGPT (units)		12				16		17		21	24	28		22	13	16	8.0	16
Alk. phos. (units)		39				8		72		98	17.9	41		54	21.0	32	22	41
CPK (units)											82				44.0		137	
Amylase (units)											42				22.0		8.0	
Lipase (units)											1.10				0.78		1.98	
Cholesterol (mg %)		384				343		271		467	593	539		519	550	470	600	537
Uric acid (mg %)		0.6				2.1		0.6		0.8		0.6		0.5	0.54	0.3	0.91	0.8
LDH (units)		284				330		402		1196		812		904		582		728
Arginase (units)											5.0				0.0		2.5	
ALT (units)		15		9		18		11		22		18		30		18		18
Calcium (mg %)		8.8				9.0		8.7		12.1	9.4	9.7		9.9	10.68	9.0	11.47	10.3
Phosphorus (mg %)		5.0				8.3		4.4		6.1	6.06	5.7		6.8	9.42	9.9	10.3	10.6
Magnesium (mg %)											1.81				3.16		2.57	
Sodium (m Eq 1)		132				133		135		153	132	135		129	121	118	121	117
Potassium (m Eq/1)		3.3				4.4		4.1		4.0	3.9	3.8		2.9	4.6	3.3	4.3	3.4
Chloride (m Eq/1)											94				86		86	
Thyroid hormones (ng/ml)																		
T3		1.74				2.02		2.98		5.03		5.96		3.23		3.23		4.91
T4		44				54		27		34		38		37		19		20
Cortisol (ng/ml)		171				189		114		74		256		63		152		657

PO - Post-oiling
(1). toxic signs and granules in granulocytes.
(2). 1+ poikilocytosis; 1+ macrocytes; 2+ anisocytosis;
1+ polychromasia Howell-Jolly Bodies; Leptocytes and Rouleaux.
(3). Howell-Jolly Bodies.

Table 6-5. HEMATOLOGY AND BIOCHEMISTRY BEAR # 4

W - VSB Winnipeg
O - R. Engelhardt

PARAMETER	Nov. 13		Nov. 16		Dec. 10		Feb. 20		Feb. 26		Feb. 29		Mar. 12		Mar. 21	
									Day 2 PO		Day 5 PO		Day 17 PO		Day 26 PO	
	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O
Total Leukocytes (X10^3 = /μl)				10.4				6.1		8.4		11.3	7.4	6.9		
Neutrophils (%)				55				74		71		84	72	73		
Bands (%)				13				2		6		8		2		
Eosinophils (%)				2				0		0		0		0		
Basophils (%)				5.0				0		0		0		0		
Lymphocytes (%)				13				24		23		10	25	25	B	
Monocytes (%)				12				0		0			3	0	L	
Metamyelocytes (%)															O	
Myelocytes (%)															O	
Other															D	
RBC (x10^6/μl)				6.8				6.22		5.56		5.75	5.42	5.25	C	4.85
PCV (%)				45				45		39		38.0	37	38.8	L	30.5
Hemoglobin (g/dl)				16.3		17.4		15.6		13.7		13.5	12.8	13.0	O	12.9
MCV (fl)				66.2				72.3		70.1		66.0	68.2	69	T	62.9
MCH (pg)				24.0				24.8		24.6		23.5	23.6	24	T	26.6
MCHC (g/dl)				36.2				34.3		35.1		35.5	28.9	34	E	42.3
Metarubricytes															D	
Other																
BUN (mg %)		7.6		6.7		19.0		11.2		7.1		10.0	13.5	13.3	112	149.5
Creatinine (mg %)		1.1		1.0		1.8		1.2		1.3		1.7	1.92	1.3	11.3	12.4
Glucose (mg %)		136		N.A.		151		221		173		187	101	178	99	78
Total Protein (gm %)		7.5		7.8		7.6		6.6		6.8		7.2	8.6	7.0	11.2	7.0
Albumin (gm %)													5.46		4.9	
α_1 – globulin (gm %)													0.78		1.0	
α_2 – globulin (gm %)													0.55		1.2	
β – globulin (gm %)													1.67		1.5	
γ-globulin (gm %)													1.05		2.6	
A/G ratio													1.13		0.76	
Bilirubin-																
Total (mg %)		0.1		0.2		0.1		0.1		0.2		0.2	1.4	8.3	15	18.8
Direct (mg %)																
Indirect (mg %)																
SGOT (units)		93		77		70		93		40		98	42	135	200	91
SGPT (units)		17		14		25		18		14		28	17	14	160	26
Alk. phos. (units)		65		36		34		105		11		25	25	75	43.2	87
CPK (units)													88		367	
Amylase (units)													5		111.0	
Lipase (units)													0.95		8.6	
Cholesterol (mg %)		274		299		367		232		252		301	464	405	600	539
Uric acid (mg %)		0.6		2.1		0.6		0.7		0.4		0.7		0.6		3.7
LDH (units)		454		418		369		632		452		396	324	984		996
Arginase (units)													2.0		100	
ALT (units)		31		29		62		18		22		29		11		18
Calcium (mg %)		9.1		9.0		7.4		9.0		9.0		9.2	11.06	8.5	14.36	11.9
Phosphorus (mg %)		4.8		6.1		4.9		4.3		5.9		5.9	6.0	4.2	8.56	13.1
Magnesium (mg %)															3.28	
Sodium (m Eq/l)		138		134		128		141		143		133	137	139	137	126
Potassium (m Eq/l)		3.7		4.5		3.4		4.6		4.2		4.2	3.3	3.3	10.9	7.8
Chloride (m Eq/l)													97	97	70	
Thyroid hormones (ng/ml)																
T3		2.5		1.76		1.45		2.35		3.76		7.32		6.63		7.99
T4		32		27		58		26		31		27		42		17
Cortisol (ng/ml)		86		35		102		62		185		300		108		2375

PO - Post-oiling
(1). toxic signs and granules in granulocytes.
(2). 1+ poikilocytosis; 1+ macrocytes; 2+ anisocytosis;
1+ polychromasia Howell-Jolly Bodies; Leptocytes and Rouleaux.
(3). Howell-Jolly Bodies.

Table 6-6. Summary of the effects of oil exposure on erythrocytic parameters, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No. 2	Bear No. 3	Bear No. 4
1st week	Hematocrit	↓	↓	
	RBC count	↓	↓	↓
	Hemoglobin	=	↓	↓
	MCV	=	=	=
	MCH	=	=	=
	MCHC	=	=	=
2nd to 5th week	Hematocrit	↓	↓↓	↓↓
	RBC count	↓	↓	↓
	Hemoglobin	↓↓	↓↓	↓↓
	MCV	↓	↓	↓
	MCH	↓	↓	=
	MCHC	↓ ,↑	↓ ,↑	↑

* ↑↑ increase
↑ possible increase
= no change
↓ possible decrease
↓↓ decrease

6.3.3. Clinical Biochemistry

6.3.3.1. Electrolytes

Sodium values in serum were determined to be in a control range of 128 - 141 mEq/l, before oil exposure in all four bears. Responses soon after exposure, in the first week, were equivocal, showing a possible decrease in Bear 2, possible increase in Bear 3, and no real change in Bear 4. From this time on, however, Bears 2 and 3 exhibited a decline in Na^+, to values approximately 15 concentration units below their individual pre-exposure normals. Bear 4 showed no marked change. During the course of treatment, Bear 2 showed recovery by mid-April.

Serum K^+ levels did not show the clear trend for decreased concentration exhibited by Na^+. Normal values were observed to be of a range from 3.2 to 4.6 mEq/l .

No changes from individual pre-exposure ranges were observed in the first week post-dosing, and none in Bears 3 and 4 for the rest of the observation time. Only Bear 2 had a decline in serum K^+, evidenced by the beginning of April. This recovered rapidly from that point on.

Calcium in serum was modified in the first week of exposure only in Bear 3, showing an elevation above its control values, and above the control range for all four bears of 8.2 - 10.4 mg/dl. This trend for an increase in serum Ca^{++} continued in the next categorized effects period, with possible elevations noted in Bear 3 and Bear 4. Bear 2 demonstrated normal Ca^{++} concentrations after April.

The electrolytic and functional partner of Ca^{++}, inorganic phosphate, was assessed to be in a control range of 4.3 - 8.3 mg/dl. No changes followed in the first week after oil exposure. The subsequent period of observation at Churchill showed an increase in the inorganic phosphate levels in all bears, from almost doubling, to a three factor increase in serum concentration. Bear 2 showed recovery to a normal range of concentration after this point.

Table 6-7. Summary of the effects of oil exposure on leukocytic parameters, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No. 2	Bear No. 3	Bear No. 4
1st week	WBC count	↑	=	=
	Seg. neutrophils	↑	=	↑
	Immat. neutrophils	=	=	=
	Eosinophils	=	=	=
	Basophils	=	=	=
	Lymphocytes	↓	↓	=
	Monocytes	=	=	=
2nd to 5th week	WBC count	↑,↓	=	=↓
	Seg. neutrophils	↑,↓	↑	↓
	Imm. neutrophils	↓	↓	=
	Eosinophils	=	=	=
	Basophils	=	=	=
	Lymphocytes	↑↓	↓ =	=
	Monocytes	=	=	=

*	
↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

The opposing changes in concentrations of Ca++ and inorganic phosphate are also evidenced by a simple calculation of Ca++/$PO_4^{\equiv}$ ratios. Normal ratios were found in control samples, ranging (with one exception) from 1.5 to 2.1. The Ca++/$PO_4^{\equiv}$ ratios did not change in the first week post-exposure, reflecting the limited changes in Ca++ and inorganic phosphate levels at this time. By the end of the experimental period at Churchill, however, the ratios were found to show a major decline to approximately one-half of normal values. Bear 2 showed a partial recovery in the ratio values during the Winnipeg holding period.

As a result of inadequate sampling data for the other two ions examined, Mg++ and Cl^-, no conclusions can be drawn as to the effect of oil exposure, nor can comparisons be made with control values. There is a tendency for Mg++ to be elevated in the 2nd to 5th week post-oiling.

A summary of electrolyte changes in serum is presented in Table 6-8, showing that, in general, major ion imbalances occurred after a latency period of at least one week post-exposure. Only K^+ concentrations appeared to be little affected.

6.3.3.2. Diagnostic Enzymes

Glumatic oxalacetic transaminase levels in serum ranged from 27 - 129 mIU/ml in the control samples. Only Bear 2 exhibited an increase, to approximately double its normal values one week after oil exposure. After that period, levels showed episodic elevations in Bears 2 and 4, at the beginning of April in the former and in mid-March in the latter. Subsequent to the early-April rises in serum GOT, levels in Bear 2 normalized again.

Another transaminase enzyme, glutamic pyruvic transaminase (GPT) showed low normal values in serum, of a range of concentrations from 10 to 52 mIU/ml. Individual bears were consistent in their own basal serum GPT levels. Serum GPT was elevated only in Bear 2 in the first week post-oil and then normalized. Towards the end of the five week post-dosing period, Bear 2 again showed an episodic rise in GPT. This normalized again. None of the other bears changed and no relevant trends could be determined. It was found that elevations in GPT did not for the most part parallel elevations in GOT as far as individual serum samples were concerned.

The third transaminating enzyme measured, alanine aminotransferase (ALT), showed no changes consistent with elevations in either GOT or GPT. The levels of ALT were not found to vary from a measured control range of 9 to 62 units/ml, at any time after the oil exposure event, in any of the bears.

Table 6-8. Summary of the effects of oil exposure on electrocytic concentrations, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No. 2	3	4
1st week	Na^+	↓	↑	=
	K^+	=	=	=
	Ca^{++}	=	↑	=
	$PO_4^{\equiv}$	=	=	=
	$Ca^{++}/PO_4^{\equiv}$	=	=	=
2nd to 5th week	Na^+	↓↓	↓↓	↓
	K^+	↓	=	=
	Ca^{++}	=	↑	↑
	$PO_4^{\equiv}$	↑	↑↑	↑↑
= $PO_4^{\equiv}$	$Ca^{++}/PO_4^{\equiv}$	↓↓	↓↓	↓↓

* ↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

Alkaline phosphatase had a wide normal plasma concentration range, both among and within individuals, at 8 to 105 mIU/ml serum. As related to individual ranges, elevations were noted in the first week after exposure in Bear 2, to three times control values, and possibly in Bear 3, approximately doubling in concentration. No further significant elevations were noted in any of the bears for the duration of the observation period.

The most marked changes in serum enzyme levels were found in lactate dehydrogenase (LDH). This enzyme was of a normal control range of approximately 300 to 600 mIU/ml. Both bears 2 and 3 exhibited elevations to two or three times, respectively, of their pre-exposure values in the first week after oiling. These two bears maintained elevated LDH levels throughout the rest of the post-dosing period at Churchill.

Bear 4 also demonstrated an LDH increase by mid-March, maintained at double its normal values. Bear 2 demonstrated a further sharp rise in serum LDH in the early-April samples to four times its normal; thereafter, concentrations declined into the control range.

The enzymes CPK, amylase, lipase, and arginase could not be correlated definitively with oil exposure. Their assay was not part of the experimental toxicity testing and thus only inadequate data is available. From such data, it may be said that only CPK showed a consistent elevation late in the post-oiling period in all three bears. Amylase, lipase, and arginase levels showed no consistent response, except for Bear 4 where a terminal elevation was noted, particularly for amylase.

It can be seen readily in the summary (Table 6-9) that only LDH exhibited consistent and major elevations in serum. Elevations in the other enzymes examined either did not occur (ALT) or were short-lived or episodic in nature (GOT, GPT, alkaline phosphatase). Thus, no clear-cut or consistent changes were noted in the other enzymes studied throughout the experimental period.

6.3.3.3 Bilirubin, Total Protein, Glucose, and Cholesterol

Bilirubin, assessed as total bilirubin, showed elevations, particularly in the later phases of the post-dosing period. Normal values were determined to be at the lower end of a 0 to 0.8 mg/dl range. In the first week after dosing, only Bear 3 showed an increase. This continued rapidly to involve all bears, exhibiting plasma levels as high as 4 to 19 mg/dl during the second to fifth week post-oil. The highest concentration was noted in Bear 4 at time of death. Therapy in Bear 2 at Winnipeg resulted in declines to near normal levels within one month.

Serum total protein was determined as a normal range of 6.2 to 7.8 g/dl. Only Bear 3 showed an increase, by approximately

Table 6-9. Summary of the effects of oil exposure on enzyme levels, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No.		
		2	3	4
1st week	GOT	↑	=	=
	GPT	↑	=	=
	ALT	=	=	=
	Alk. Phos.	↑↑	↑	=
	LDH	↑↑	↑↑	=
2nd to 5th week	GOT	=,↑	=	↑
	GPT	=,↑	=	=
	ALT	=	=	=
	Alk. Phos.	=	=	=
	LDH	↑↑	↑↑	↑↑

* ↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

30% in the early post-dosing period. Thereafter, both Bears 2 and 3 showed marked continued increases, in the case of Bear 2 nearly doubling in serum total protein by the last week in March. Bear 4 showed no relevant protein changes. Recovery was rapid in Bear 2 from the beginning of April on. There was no consistency in the recorded elevations in serum enzymes and the pattern of protein elevation.

Serum glucose, observed in a normal control range of 115 to 286 mg/dl, wide overall and within individuals, showed generally no oil-related response. Bear 4 did show a terminal decrease at the time of death.

Cholesterol in serum was observed to show variable response in the first week post-oil dosing. Elevations, depressions, and no change was found as related to individual control ranges. Overall, a normal control range of 232 to 392 mg/dl was found for the four bears. Cholesterol values were elevated in all three oiled bears, however, throughout the second to fifth week post-oiling period. Double the normal concentrations were determined. From April on, Bear 2 again became normalized in serum cholesterol concentration.

The results of the diverse assays described in this sub-section are summarized in Table 6-10.

6.3.3.4 Nitrogenous End-Products of Metabolism

Urea nitrogen was found to exhibit major changes after oil exposure. A normal control range for the four bears of 4 to 33 mg/dl was established. Soon after oil exposure, a small decline in urea nitrogen occurred in Bears 2 and 3. More obvious were the major rises occurring towards the end of March, with values of approximately 150 mg/dl general. These coincided with the day of death in Bear 3 and Bear 4. Treatment of Bear 2 resulted in a recovery to normal serum urea nitrogen by July.

Creatinine in serum showed a similar pattern of changes from control values of 0.7 to 2.5 mg/dl. Terminal elevations to nearly 13 mg/dl were observed in Bears 3 and 4. Bear 2, with values nearly as high at the same time, recovered by the end of May.

Uric acid levels, however, showed a less clear response to oil exposure. A normal range of 0.5 to 2.1 mg/dl was determined, which was not exceeded in the early post-oil period. By the end of the Churchill experimental phase, Bears 2 and 4 had elevated serum uric acid. This subsequently normalized rapidly in Bear 2. No such finding was noted with Bear 3.

The summary of changes in nitrogenous waste products emphasizes major latent elevations in urea nitrogen and creatinine (Table 6-11).

Table 6-10. Summary of the effects of oil exposure on serum levels of bilirubin, protein, glucose, and cholesterol, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No.		
		2	3	4
1st week	Total bilirubin	=	↑	=
	Total protein	=	↑	=
	Glucose	=	=	=
	Cholesterol	↓	↑	=
2nd to 6th week	Total bilirubin	↑↑	↑↑	↑↑
	Total protein	↑↑	↑	=
	Glucose	=	=	=, ↓
	Cholesterol	↑↑	↑↑	↑↑

* ↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

Table 6-11. Summary of the effects of oil exposure on serum levels of nitrogen compounds showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No.		
		2	3	4
1st week	Urea nitrogen	↓	↓	=
	Creatinine	=	=	=
	Uric acid	=	=	=
2nd to 5th week	Urea nitrogen	↑↑	↑↑	↑↑
	Creatinine	↑↑	↑↑	↑↑
	Uric acid	↑↑	=	↑

* ↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

6.3.4 Plasma Hormones

Cortisol levels in plasma were highly variable, but nonetheless showed a particular elevated response after oil exposure. The variablility included the total control range, measured to extend from 35 to 275 ng/ml, variable also within individuals to almost the same degree. Two of the bears, Bear 2 and Bear 3, show an apparent decrease in their plasma cortisol concentration in the first week after oil exposure, while Bear 4 shows an increase. All three bears, however, show further increased cortisol during the second effects period, extending to the end of March. The elevations were sporadic among the samplings, however. Bears 3 and 4 showed the highest observed plasma cortisol on the days of their death. Bear 4 at this time exhibited a phenomenally high serum cortisol level at over 2 thousand ng/ml. Bear 2 continued to display elevated plasma cortisol levels during its stay at the Assiniboine Zoo, which did not decline in concentration to normal levels until July.

The responses of the two thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) were not similar. Control levels of T_3 were of a range of 1.5 to 3.7 ng/ml. Elevations to approximately double individual control values resulted in the first week after exposure. This increase was generally maintained subsequently, and reached levels as high as three to four times control values during the period at Churchill. The greatest elevations in Bears 3 and 4 were observed terminally. Concentrations of T_3 in Bear 2 normalized rapidly with treatment.

The picture varied for T_4 levels. No departure from normal values of 22 to 58 ng/ml were found in the first week post-oil. Only late in the second period, by about five weeks post-oil, was there a decline of T_4 in plasma, going below control levels in all three bears. As before, Bear 2 normalized rapidly with treatment.

A summary of plasma hormone changes is presented in Table 6-12. Major changes are evident in later stages after the oiling event, and there may be a reciprocal relationship between high cortisol levels and thyroxine.

6.4 Discussion

6.4.1 Assessment of Exposure

Considering all possible routes of petroleum hydrocarbons uptake in the experimental polar bears, ingestion and absorption through the gastrointestinal tract appears to be the most significant route. Inhalation probably contributed to the total hydrocarbon uptake and absorption through the skin could have occurred, especially in the heavier oiled bears which had more oil present at the skin surface.

Table 6-12. Summary of the effects of oil exposure on levels of plasma hormones, showing relative changes*, as compared to values prior to the exposure event.

Time Post-Exposure	Parameter	Bear No.		
		2	3	4
1st week	Cortisol	↓	↓	↑
	Triiodothyronine	↑	↑	↑
	Thyroxine	=	=	=
2nd to 5th week	Cortisol	↑↑	↑↑	↑↑
	Triiodothyronine	↑	=, ↑	↑↑
	Thyroxine	=, ↓↓	=, ↓↓	=, ↓↓

* ↑↑	increase
↑	possible increase
=	no change
↓	possible decrease
↓↓	decrease

Crude oil hydrocarbons and more defined and concentrated crude oil fractions uptake has been studied in various mammals. Engelhardt *et al*. (1977) reported rapid absorption of hydrocarbons from Normal Wells crude oil into body tissues and fluids of ringed seals (*Phoca hispida*) when exposed by both immersion and ingestion. According to Bratton *et al*. (1975), charcoal lighter fluid or naphtha which is a more volatile hydrocarbon mixture than kerosene is not appreciably absorbed from the gastrointestinal tract of rats. Mann *et al*. (1977) found that primates, however, absorb kerosene from the gastrointestinal tract, but the amount absorbed is small. The uptake of toluene, which represented the most volatile fraction of the kerosene distillate, was greater than that of hexadecane. Rats, rabbits, and dogs were also mentioned in this report as species in which kerosene absorption from the gastrointestinal tract occurs in amounts sufficient to cause symptoms. Gerarde (1959), demonstrated that aromatic hydrocarbons present in a kerosene were absorbed rapidly into blood from the gastrointestinal tract in rats, rabbits and white Leghorn hens. Hydrocarbons absorbed from the G.I. tract after ingestion of gasoline caused symptoms in a child, and xylenes and naphthalenes in the gasoline were implicated as the causative agents (Alder *et al*. 1976). Rapid absorption of gasoline hydrocarbons was indicated in another report of childhood poisoning by Stockman, (1977). Zieserl (1979), summarized some experimental studies and concluded that petroleum hydrocarbons are absorbed from the G.I. tract, but in very small amounts. Aromatic compounds are absorbed in much larger proportions than aliphatic compounds.

Vapor-phase petroleum hydrocarbons are absorbed rapidly and at high levels into blood from the pulmonary alveoli. The amount absorbed is directly related to the rate and volume of air exchange and would therefore be higher during exercise (Committee on Medical and Biological Effects of Environmental Pollutants, 1976). Percutaneous absorption of lipid-soluble hydrocarbons occurs via the skin lipids, hair follicles and sebaceous glands. The rate of absorption depends on the rate of blood circulation through the skin, (Tagami et al. 1973). According to Hunter (1968) enough gasoline was absorbed through skin in a case of extensive skin exposure for renal damage to occur.

The crude oil hydrocarbon uptake in this study was both of an acute and chronic nature. Initial acute exposure included mainly ingestion. The polar bears began licking the oil off the fur immediately after leaving the oil tank and engaged in this activity most vigorously in this immediate post-oiling period when the fur was the heaviest laden with oil. However, the bears also vomited on several occasions during this period and thus the ingested oil was lost. How much oil passed into the intestine between the vomitings and how much of the more volatile fractions could have been absorbed through the stomach wall is not known, but it is evident that it did enter the body (see Chapter 5).

Pathological exposure is likely to have also occurred through inhalation and skin absorption, especially of the more volatile hydrocarbon fractions.

Smaller amounts of the crude oil with most likely a different composition of hydrocarbons were ingested during additional grooming in the pre- and post- period. From the available information it was not possible to arrive at any even approximate estimation of the amount of the crude oil hydrocarbon uptake via the various probable routes. Therefore the limits of sensitivity of this animal species to this particular crude oil are still an open question.

There were also a variety of factors that may have influenced the animals' response to the crude oil. Complex relationships may exist between the nutritional status and the toxic agent(s). Dieting factors can produce changes in the body composition, physiological and biochemical functions and nutritional status of the animal and thereby influence toxicity (Doull 1980). The experimental diet could not be evaluated in this light, due to lack of information on its adequacy. The experimental animals on this diet maintained their body weight in two cases, and one bear experienced weight loss in the pre-oiling period (see Appendix 5). It should be noted, however, that this bear appeared to be quite fat at capture.

Direct injury to an organ prior to exposure can be another means of increasing toxicity of a compound(s). The drugs used for immobilization, ketamine HCl and xylazine HCl, have been used in various species and there is no indication that these drugs cause primary renal or hepatic damage. However, they are contraindicated in advanced hepatic and renal disease (Short 1974, Booth 1977, Spinelli *et al*. 1978).

6.4.2 Hematotoxicity

All three bears developed a toxic anemia which was normocytic and normochromic in the first week after exposure and progressed to a serious microcytic and hypochromic toxic anemia by the end of March. The anemia seems to have developed fron an arrest of hematopoiesis. No recovery is evident since MCV and MCH do not show an increase (Schalm *et al*. 1975). Some recovery in hemoglobin synthesis shows itself near the end of the experimental observation period.
It is interesting to note the moderate anemia in the unoiled Bear 1. Similar degrees of anemia were present in two other bears at about the same period. Whether the apparent anemia was due to the crude oil hydrocarbon inhalation from the circulating air in the L-9 building or related to something else, could not be determined.

Bone marrow depression has been seen in benzene poisoning which is a known hematopoietic toxin (Arena 1979). Changes in erythrocyte parameters were most likely due to cell membrane alteration by the crude oil hycrocarbons, either by direct action on the membrane lipids, or by modifying the biochemical mechanisms responsible for membrane maintenance with subsequent volume changes from dehydration and probably splenic action. It is possible that cell volume and hemoglobin contents were altered at the bone marrow level at some stage post-oiling, however the bone marrow differential count (Chapter 7) indicated erythropoietic arrest (probably reversible) at the stem cell level at the time of autopsy. The severe petroleum-induced anemia fits with the reported actions of benzene and naphthalene on blood (Truhaut and Bohnon 1963, Villani *et al*. 1960, Perrelli *et al*. 1970).

In contrast to our findings, the study of oil ingestion and immersion by ringed seals, using a lighter Norman Wells crude oil, was not found to have hematological effects (Smith and Geraci 1975, Geraci and Smith 1976). The studies are not strictly comparable, however, since the amount of oil ingested by the bears was undoubtedly greater, with a more acute dose, and also a longer dosing period as a result of grooming intake. Perhaps longer dosing in the seals would have had similar effects.

Control values obtained from these bears form an upper range of what are reported to be field values for polar bears by Lee *et al*. (1977) and Seal *et al*. (1967), and were comparable to other reported results (Wallach 1978, Schalm *et al*. 1975). There was some pre-oiling drop in erythron values that most likely represented adaptive changes to captivity. Similar changes occur in captive marine mammals due to decreased oxygen demand (Medway and Geraci 1978).

The hemolysis evident soon after oil exposures is in keeping with the increase in serum bilirubin starting at that time. This increase continues to show a drastic rise by the end of March when a renal insufficiency complicates the situation further. A decreased liver function is not indicated at any but the earliest times of post-oil observation. This strengthens the suggestion of a terminal renal failure as a cause for high bilirubin.

Mention must be made of the increase in plasma protein after oil exposure. This normally very constant parameter may indicate a dehydration in the bears, a likely possibility since their food and water intake was lessened at that time and they were experiencing additional fluid loss by diarrhea. Correction of erythrocytic parameters by a dehydration index based on serum protein normalcy would further strenghten the already evident anemia.

With regard to white cell parameters, few changes could be attributed to the effect of oil exposure on total white blood cell count. The alterations noted by Pawelski *et al*. (1964, 1965), Girard *et al*. (1970) and Friborska (1973) were not seen in the bears. The bears do not seem to be as highly susceptible to abnormalities in leukocytic functions as they are in erythrocytic ones in response to petroleum. Some shifts did occur in neutrophil/lymphocyte ratios, which are more likely a consequence of cortisol fluctuations than a direct oil related effect. A recognized cortisol action is to depress lymphocytes, and immune responses, giving rise to a proportional increase in neutrophils.

The myelocytic portion of bone marrow, giving rise to white blood cells, was not affected. This would be seen readily since white blood cells have a much shorter residence time than do erythrocytes. It also suggests that the oil caused a biochemical lesion in the synthesis of erythrocytes, since the high levels of hydrocarbon residues noted in the marrow would otherwise have affected both red and white blood cell precursors by a direct action.

Again, white blood cell parameters of the control bears in this study, Bears 1, 2, 3 and 4, were in the range of normal field values reported by Lee _et_ _al_. (1977) and Seal _et_ _al_. (1967).

6.4.3 Electrolyte Balance

Electrolytes, of normal values before the start of the experiment according to the comparative references, showed major imbalances in Na^+, and PO_4 in the progression of the post-oiling period. A primary renal problem is suggested, since no adrenocortical insufficiency was indicated. Cortisol levels stayed high. While Ca^{++} levels stay constant, or nearly so in serum, altering the $PO_4^{\equiv}$ ratio to approach unity. A problem in acid-base balance at the level of the kidney is suggested.

Serum Na^+ and $PO_4^{\equiv}$, initially in the normal range of values reported by Lee _et_ _al_. (1977) and Manery _et_ _al_. (1966), definitely move out of _this_ range as a consequence _of_ the oil contamination. Even though major RBC lysis occurs at this time, there is no major contribution to the serum K^+ pool, as may be expected in such circumstances in many other mammals, including the human. The polar bear has comparatively low K+ concentrations in its erythrocytes, isotonic with serum (Manery _et_ _al_. 1966).

6.4.4 Serum Enzymes

The profile of the reputedly hepatotoxicity-specific enzymes assayed in this study does not indicate any long-lived hepatic effect, even though the bears carried some oil throughout the study in body fluids and tissues. Only initially, at times of maximum exposure, were there any conclusive signs of hepatotoxicity. The exposure at the time immediately after dosing was also to a mixture of hydrocarbons, which contained appreciably more low molecular weight hydrocarbons, including benzene, than were present later on. It is only these compounds which have been referenced as having some, even though limited, hepatotoxicity (Rozera 1960, DiBlasi _et_ _al_. 1964).

Although serum LDH does show elevations in the polar bears, this occurs predominantly in the later post-oiling stages. The LDH elevations are not isozyme-defined, and are more likely of hemolysis origin, since erythrocytes commonly contain one LDH isozyme pattern. Nonetheless, LDH was identified as showing increased serum levels after benzene poisoning (Lob 1963) and the early elevations in LDH, as well as in alkaline phosphatase, may be correlated; that is, responsive to the low molecular weight fractions in the fresher oil.

Serum levels of GOT, GPT, LDH, and alkaline phosphatase in the control samples compare positively with the values for normal field bears for these enzymes reported by Lee et al. (1977). Additionally, all five enzymes assayed were in the low to normal range of values as compared to their concentrations in serum or plasma of a wide spectrum of mammals (Altman 1961).

Following exposure of ringed seals to oil, Geraci and Smith reported a low order transient rise in diagnostic liver enzymes, similar to what was found in this study. In neither case was hepatotoxicity apparently a major debilitating factor.

6.4.5 Cholesterol

Cholesterol in serum, which initially was in the range of values described by Lee et al. (1977), rose towards the most critical period in the health of the bears at the end of March. This increase recalls the findings that in laboratory mammals and human studies there was a rise in plasma cholesterol and increased cholesterol synthesis in response to contamination by gasoline and by n-paraffins (Abdullaev et al. 1971, Pokrovskii et al. 1973, Mekhtier 1966).

6.4.6 Renal Function

Evidence of kidney dysfunction has already been presented in the major rise noted in bilirubin, and decline in serum Na^+, at the end of March. Further support for a massive kidney failure at this time in all three bears is found in marked elevations in urea nitrogen, creatinine, and uric acid, as compared to control values prior to oiling and also for urea levels, to Lee et al. (1977).

Although the kidneys showed no major accumulation of oil residues, their direct exposure level must have been high and prolonged. Urine hydrocarbon values remained elevated throughout the experimental period. It is then perhaps not surprising that this was one of the major tissues affected.

6.4.7 Hormone Balance

The capacity of the bears to respond with major increases in the hormone at the end of the experimental period points out that there was no exhaustion of steroid reserves in the adrenal cortex. Decreases noted in the first 2 to 3 weeks after oiling, however, are suggestive of some negative balance. This decrease has also been described by Kanner (1963), DiBlasi (1965), Makotchenko (1971), and Kashkin et al. (1968). Cortisol values in the bears are higher than the normal unstressed mammalian range of approximately 20-40 ng/ml (Altman 1961), found also in phocid seals (Engelhardt and Ferguson 1980).

A hydrocarbon-stimulated increase in thyroid secretion activity has been found by Iannaccone and Cicchella (1959). With regard to T_3, a similar situation exists in the bears but not with regard to T_4 hormone, which shows a decline towards the end of the experimental period. Since the T_3 hormone is generally considered to be the active hormone (Kutsky 1973), there is a consideration necessary about its stimulatory effects on heat generation and metabolism examined by Øritsland and colleagues. In a comparative study on thyroid function in mammals, Joasoo et al. (1975) describe fractional thyroid hormone inter-relationships in polar bears. They determined a T_4 level of 18 ng/ml, comparable but somewhat lower than what was found in this study by radioimmunoassay.

6.4.8 Recovery

Bear 2 arrived at the Assiniboine Zoo in Winnipeg at the beginning of April in a condition nearly as debilitated and close to death as Bear 3 and Bear 4. Through the efforts of veterinary and zoo staff, the bear began a slow recovery which normalized by mid-July basically all of the parameters which had become deviated as a result of the oil exposure. The details of the health care and recovery are described in detail in Appendix 6.

6.5 Acknowledgements

We would like to thank D. Villeneuve and A. Yagminas of the Health Protection Branch, Chemical Toxicology Division, Health and Welfare Canada for the SMA-12-60 assays. D. Peakall of the Wildlife Toxicology Division of the Canadian Wildlife Service is gratefully acknowledged for his critical review of this section. The personnel of the Veterinary Services Branch, Department of Agriculture, Winnipeg, Manitoba is also thanked.

7. PATHOLOGY

F.A. Juck

7.1. Introduction

Assessment of gross and histopathology was necessitated in this study by the death of two of the three oiled bears, unexpected in the design of the exposure study. A detailed necropsy was carried out to determine causative factors, and to correlate tissue damage with clinical pathology findings described in Chapter 6 of this report.

7.2. Materials and Methods

Necropsy on Bear 4, which died at Churchill, was performed by the polar bear researchers there. Formalized organ samples used in the microscopic study were collected at that time. Photographs of the live bears, Bear 4 in the immediate post-oiling period, and Bear 2, fully recovered from the effects of oil, came from the PBCC files. Photographs taken during autopsy of Bear 4 were supplied by the polar bear researchers. The remaining photographs were taken throughout the autopsy of Bear 3. Photographs were also used to illustrate the histopathological findings.

Cerebrospinal fluid (CSF) was collected from Bear 3 soon after euthanasia. Routine bacteriological examination was done on samples obtained at autopsy of Bear 3. Routine detailed macroscopic examination of all organs was carried out immediately after euthanasia of Bear 3 and thin slices of organs were collected into 10% neutral pH buffered formalin. The brain was fixed in one piece. Bouin's fixative was used for collection of the eyes and the gastrointestinal tract for histophathological examination.

Well fixed tissues were trimmed, routinely embedded in paraffin, sectioned at 6 µm and stained with hematoxylin and eosin (H & E) for histopathological examination. Special stains were used where indicated. The following tissues were examined from both polar bears: the brain and the spinal cord at various levels, sciatic nerve, cauda equina, skin from various areas of the body, including radiotransmitter implantation sites, both eyes with conjunctivae and associated glandular tissue, the tongue, oesophagus, stomach from various areas, duodenum, jejunum, ileum, colon, pancreas, various liver lobes, gall bladder, parotid and submandibular salivary glands, nasal passages (external nares only in Bear 4), A hydrocarbon-stimulated increase in thyroid secretion activity has been found by Iannaccone and Cicchella (1959). With regard to T_3, a similar situation exists in the bears but not with regard to T_4 hormone, which shows a decline towards the end of the experimental period. Since the T_3 hormone is generally considered to be the active hormone (Kutsky 1973), there is a consideration necessary about its stimulatory effects on heat generation and metabolism examined by Øritsland and colleagues. In a comparative study on thyroid function in mammals, Joasoo et al. (1975) describe fractional thyroid hormone inter-relationships in polar bears. They determined a T_4 level of 18 ng/ml, comparable but somewhat lower than what was found in this study by radioimmunoassay.

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Necropsy on Bear 4, which died at Churchill, was performed by the polar bear researchers there. Formalized organ samples used in the microscopic study were collected at that time. Photographs of the live bears, Bear 4 in the immediate post-oiling period, and Bear 2, fully recovered from the effects of oil, came from the PBCC files. Photographs taken during autopsy of Bear 4 were supplied by the polar bear researchers. The remaining photographs were taken throughout the autopsy of Bear 3. Photographs were also used to illustrate the histopathological findings.

Cerebrospinal fluid (CSF) was collected from Bear 3 soon after euthanasia. Routine bacteriological examination was done on samples obtained at autopsy of Bear 3. Routine detailed macroscopic examination of all organs was carried out immediately after euthanasia of Bear 3 and thin slices of organs were collected into 10% neutral pH buffered formalin. The brain was fixed in one piece. Bouin's fixative was used for collection of the eyes and the gastrointestinal tract for histophathological examination.

Well fixed tissues were trimmed, routinely embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (H & E) for histopathological examination. Special stains were used where indicated. The following tissues were examined from both polar bears: the brain and the spinal cord at various levels, sciatic nerve, cauda equina, skin from various areas of the body, including radiotransmitter implantation sites, both eyes with conjunctivae and associated glandular tissue, the tongue, oesophagus, stomach from various areas, duodenum, jejunum, ileum, colon, pancreas, various liver lobes, gall bladder, parotid and submandibular salivary glands, nasal passages (external nares only in Bear 4), larynx-pharynx with epiglottis, trachea, bronchi and all lung lobes, retropharyngeal, submandibular, axillary and popliteal lymph nodes, spleen, bone marrow (from a vertebral body, Bear 3, and from an unidentified site, Bear 4), diaphragmatic, psoas, masseter and quadriceps femoris muscles, subcutaneous and visceral fat, adrenal glands, kidneys, testicles, aorta, right and left atria and the heart ventricles at various levels. The popliteal lymph node from Bear 4, and the trigeminal nerve ganglia, pituitary and thyroid glands, penis, the abscess wall, mesenteric and external inguinal lymph nodes and the rectum from Bear 3 were also examined at the light microscopy level.

A variety of tissue sections collected from thirteen normal polar bears, was reviewed as reference material for the histological study. The organ samples were collected from polar bears at various ages and both sexes that were destroyed around Churchill, Manitoba, for safety reasons, in the fall of 1976.

7.3 Results

7.3.1. Gross Autopsy Findings

7.3.1.1. Bear 3

The bear was in a moderate to poor bodily condition. It was poorly muscled and there was only a small amount of subcutaneous and intra-abdominal fat present.

Superficially, the hair coat appeared reasonably but variably clean. A fair amount of oil, however, was still present at the skin level and also the skin surface was covered with oil (Figure 7-4). The posterior neck area and shoulder hair contained more oil. Oil covered the epidermis, especially along the back, and had a fine crusty appearance that was most evident in a large almost completely hairless patch along the midback. The hair could be pulled out very easily from this area that was about 30 cm in diameter, and only sparse guard hair remained (Figure 7-5). The hair could not be pulled out from other areas of the body.

The paramedian abdominal incision site (deep body temperature radiotransmitter implantation site) was clean with no sutures present. The capsule surrounding the radiotransmitter was pale, pink and thin, and no exudate was present. The skin temperature radiotransmitter recorder site in the lateral neck area was also free of exudate. There was a small to moderate volume of moderately opaque exudate present in the left rump subcutaneous radiotransmitter implantation site. The inner surface of the implantation site capsule was moderately congested. The skin over the radiotransmitter was locally necrotic. Sutures, crusty exudate and yellow granular

Figure 7-1.

Demonstrates presence of a moderate amount of oil close to the skin, and a moderate discoloration of the hair in Bear 2.

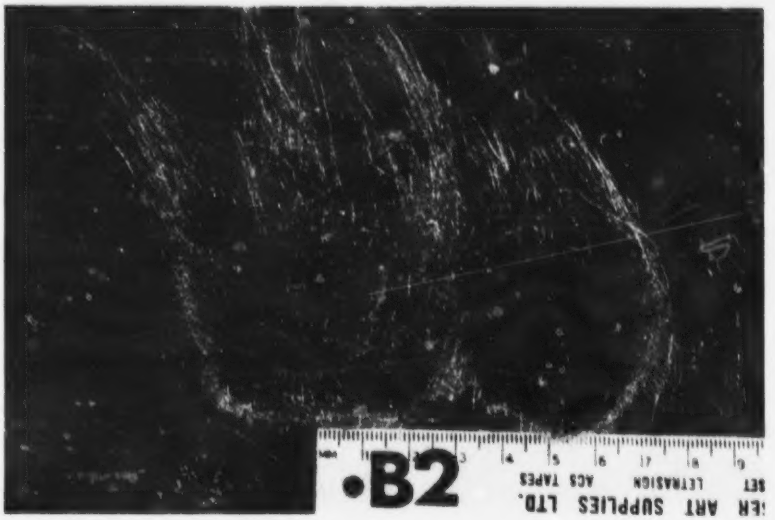

Figure 7-2.

Fully recovered Bear 2 with clean healthy hair coat.

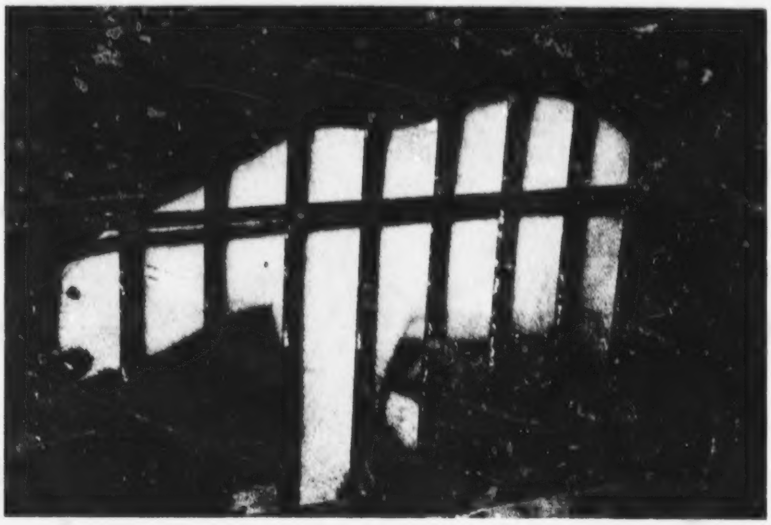

Figure 7-3.

Clinical appearance of Bear 4. The animal was depressed, lethargic and seldom moved from the sawdust box.

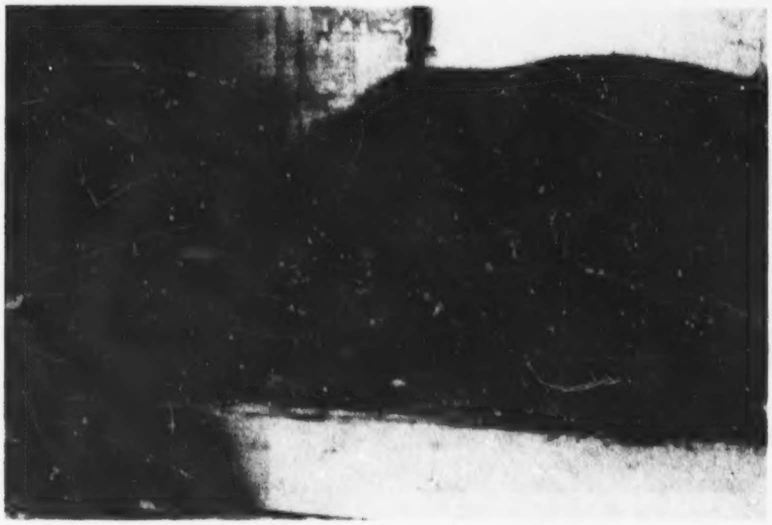

Figure 7-4. The hair is discoloured and a fair amount of oil is still present at the skin level of Bear 3.

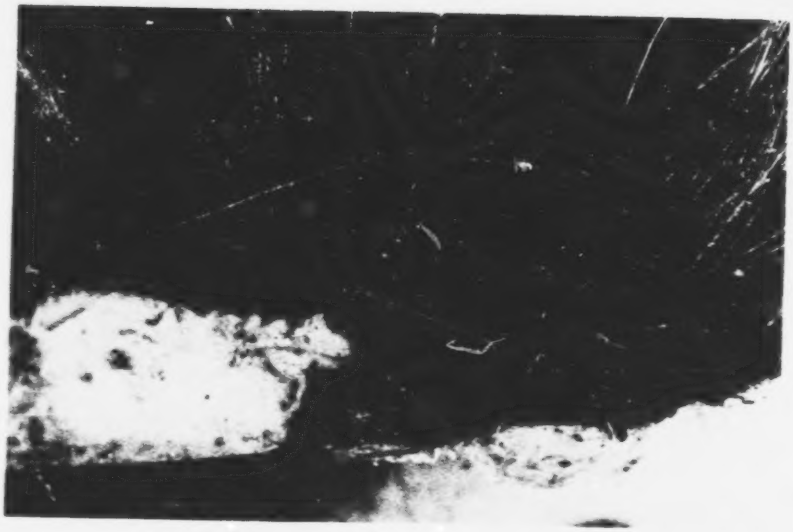

Figure 7-5. Alopecia and crusty appearance of skin from the mid-back area, Bear 3.

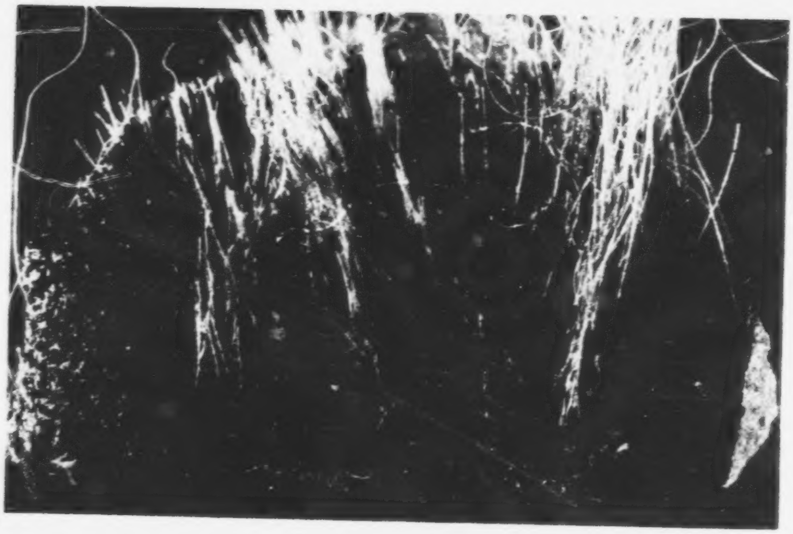

Figure 7-6. Reddish gray purulent exudate and necrotic fat masses from left lateral thigh area abscess, Bear 3.

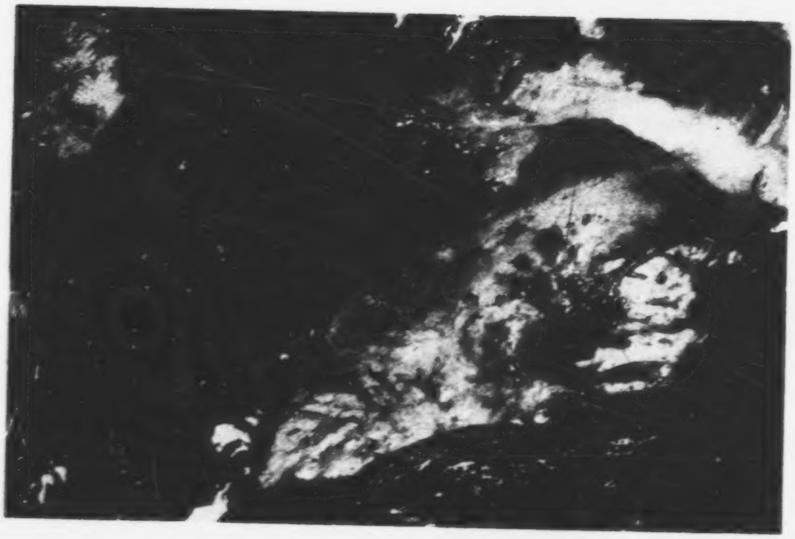

Figure 7-7.
Dirty yellow mucopurulent exudate is present at the anterior aspect of the maxilloturbinates. Yellowish inflammatory foci can be seen in the turbinate mucosa, Bear 3.

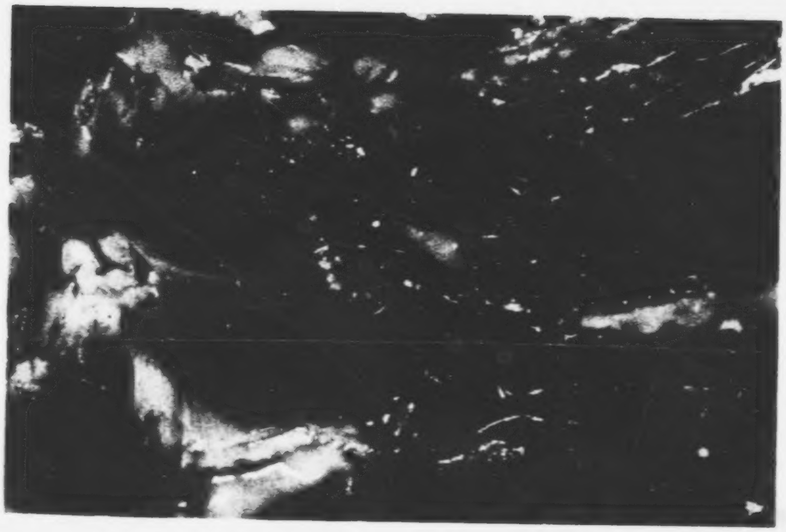

Figure 7-8.
Demonstrates necrotizing lesion on the lateral aspect of the tongue, Bear 3.

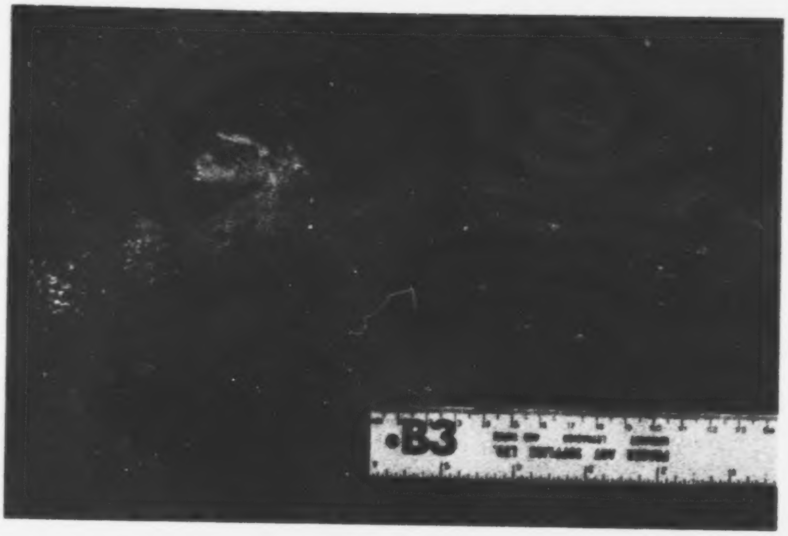

Figure 7-9.
Large volume of digested blood is present in the stomach content, Bear 3.

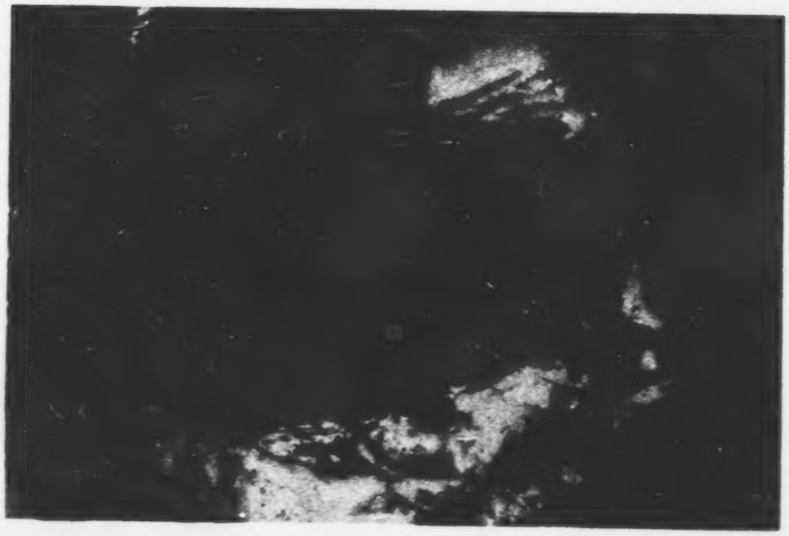

Figure 7-10. There are tiny hemorrhages and larger blood accumulations on the stomach mucosa, Bear 3.

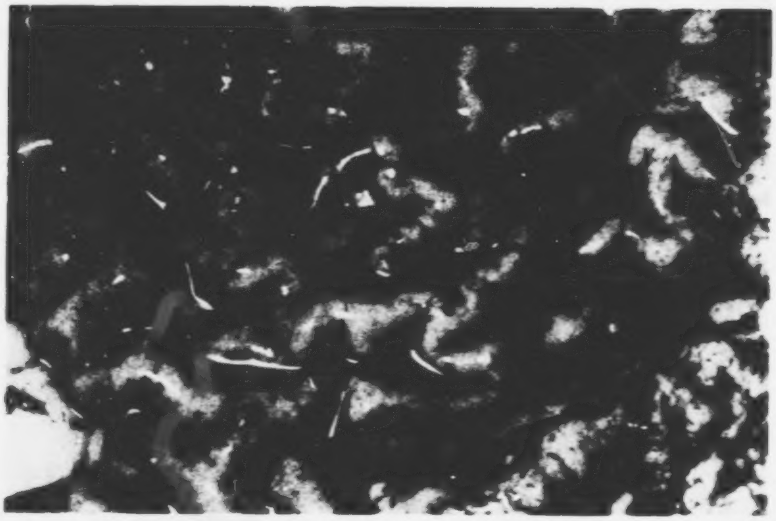

Figure 7-11. The renal cortex is of light brown colour, and fine sand-colour mottling and speckling can be seen, Bear 3.

Figure 7-12. Yellow discoloration of spinal cord in Bear 4, can be seen.

material (Furazolidone powder) were present at the two incision sites, neck and rump areas.

There was a large abscess in the subcutis of the left lateral thigh area. The reddish-gray purulent exudate contained large necrotic fat masses (Figure 7-6). The abscess capsule was very thin and friable.

A moderate amount of dirty yellow very putrid mucopurulent exudate was present in the nasal passages at the anterior aspect of the maxilloturbinates and also small yellowish foci were seen in the mucosa of the turbinates, (Figure 7-7).

There was a bilateral, fairly symmetrical, necrotizing lesion on the lateral aspect of the tongue. The tongue was most affected approximately at the level of the last premolar and first molar teeth. Yellow, rather dry debris accumulated in the tongue lesion at this level (Figure 7-8).

The stomach contents were a mixture of a large volume of brown-black flecky material (digested blood), a larger amount of hair and yellowish fluid, (Figure 7-9). There were numerous tiny brownish-red hemorrhages and larger blood accumulations on the stomach mucosa (Figure 7-10). An increased amount of mucus, mixed with globs and strands of digested blood, was evident throughout the intestinal tract. Terminally, the bear voided a small volume of oily dark feces.

The gall bladder was moderately full of moderately viscid, dark bile.

The kidney lobes were of normal size and light brown colour with fine sand-colour mottling and speckling in the cortices (Figure 7-11).

The lungs were partially atelectatic and readily collapsed with handling.

A moderately heavy growth of E. coli and Proteus sp. was obtained from the nasal exudate. There was a light growth of E. coli from the rump radiotransmitter implantation site. No bacterial growth was obtained from the subcutaneous abscess.

A cerebrospinal fluid (CSF) sample was collected at the time of necropsy and results are included in Table 7-1. Results of bone marrow cytology are presented in Table 7-2.

TABLE 7-1

BEAR 3

CSF Examination - March 25

PARAMETERS	RESULTS
Colour	Slightly bloody
Turbidity	4+
Sp. gravity	1.015
Coagulation	
Cell count	110 cells/cmm
Protein	100 mg %
Glucose	neg.
Creatine phosphokinase (IU/l)	1084

TABLE 7-2

Bone Marrow Differential - Bear 3

PARAMETERS	RESULTS
Blasts %	
Promyelocytes %	1
Myelocytes %	4
Metamyelocytes %	6
Band cells %	8
Polymorphs %	20
Eosinophils %	14
Basophils %	
Monocytes %	1
Lymphocytes %	33
Rubriblasts %	
Prorubricytes %	
Rubricytes %	3
Metarubricytes %	10
R.E. Cells	
Unclassified	

7.3.1.2. Bear 4

The mucous membranes, adipose tissue, various organs and the brain, and spinal cord, (Figure 7-12) were markedly yellow. The stomach contained a larger volume of dark brown fluid, most likely digested blood. There were tiny hemorrhages from the stomach mucosa. The right axillary lymph node examined as a fixed specimen was markedly enlarged, hemorrhagic and necrotic.

7.3.2 Histopathology

7.3.2.1 Reference Polar Bears

The organ samples of the reference animals were collected at variable times after death and this was reflected in the degree of autolysis present. A smaller number of organs were sampled and only the better preserved ones were included in this study for comparative purposes. No lesions were seen in the majority of the organ samples. There were tiny fibrotic foci and lymphocyte and plasma cell accumulations, and an occasional glomerulus was fibrotic in the kidney samples of three bears. Calcium deposits were observed in the renal papillae and granular brownish material accumulations in the convoluted tubules in the kidney sample of one bear. Sarcosporidial cysts were seen in muscle samples from several bears. Cysts with remnants of Trichinella spiralis larvae, and some giant cells and mononuclear leukocytes were present in the diaphragmatic muscle of one bear.

The cells of zona arcuata (glomerulosa) of the adrenal cortices were tall, columnar, and pallisading along the thin connective tissue stroma. The cytoplasm of the cells was deep eosinophilic, vacuolated and scanty (Figure 7-13). There was a distinct separation line from the zona fasciculata. The zona fasciculata cells were irregularly round and had finely vacuolated cytoplasma. Average ratio of zone arcuata to fasciculata and reticularis was 1:15, measured at the lesser diameter of several adrenal glands. The thickness of zona arcuata was fairly uniform around the circumference of the glands.

7.3.2.2. Bear 3

An occasional dead neuron was seen in the cerebral cortical area and an occasional sporozoan cyst was encountered throughout the brain. The brain meninges contained a few mononuclear leukocytes and some macrophages. Degenerated and necrotic cells were scattered in the small cell pyramidal layer of the hippocampus. There were a few plasma cells and a moderate number of granulocytes in the interstitium of the third eyelid gland. The conjunctival epithelium was vacuolated and infiltrated by a small to moderate number of granulocytes.

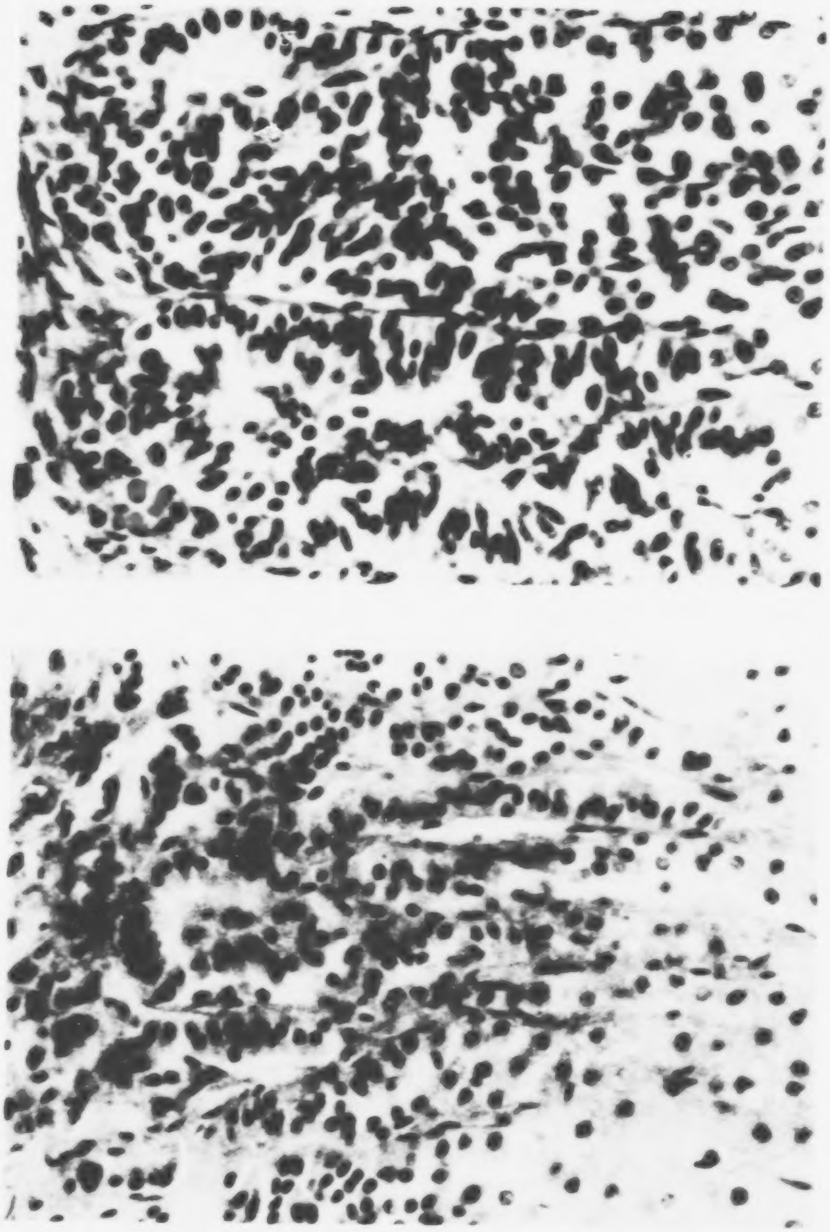

Figure 7-13. Appearance of zona arcuata and part of zona fasiculata in two reference polar bears. Note the pallisading arrangement of cells and a small amount of cytoplasm. Adrenal capsule to the left. Magnification 3750 x.

The zona arcuata varied in thickness between 1/2 to 2 times the normal size. The pallisading pattern of the tall, thin, columnar cells was also variably preserved. Some of the arches had fewer cells and the cells were enlarged, irregularly round and vacuolated (Figure 7-14). The approximate ratio of zona arcuata to fasciculata and reticularis was 1:20.

A large proportion of the renal tubules were moderately to markedly dilated and contained hemoglobin or a mixture of hemoglobin and brownish pigment indicative of hemoglobin degradation products. The hemoglobin pigment was either free in the tubular lumina as loose granular casts or was associated with sloughed epithelial cells (Figure 7-15). The bilirubin pigment was either free in the lumina as large, variable-sized granules, alone or mixed with hemoglobin, or as numerous fine granules in the cytoplasm of the renal epithelial cells. Some tubules contained numerous degenerated epithelial cells, and granulocytic exudate was seen in others (Figure 7-16). In less affected tubules, the epithelial cells were vacuolated, and stretching of epithelial cells occurred in tubules with epithelial cell loss. A moderate number of oxalate crystals was seen in the tubules. Fine calcium precipitate was present in slightly edematous renal interstitium and around the tubular basement membranes. The glomerular capillaries were slightly thickened and the mesangial cells were prominent. There was also thickening of the basement membranes of Bowman's capsules and the epithelial cells were prominent. There were some interstitial hemorrhages in the renal papillae and some degeneration and granulocytic infiltration of the papillary epithelium. The renal pelvis contained hemoglobin casts and free blood. Small lymphocytic accumulations were seen in the peripelvic interstitium.

The seminiferous tubules were not patent and contained an occasional degenerated spermatogonium. Granulocytic exudate and cellular debris occupied an occasional epididymal duct.

The skin from the neck area was normoplastic but hyperkeratotic, and the hair follicles were distended with cornified epithelial cells. The hair follicles contained fewer hair shafts. The skin from the dorsal alopecic area had moderately to markedly hyperplastic with a markedly hyperkeratotic epidermis. The hair follicles contained occasional hair shafts and were distended with cornified epithelial cells. The apocrine glands were moderately dilated and the gland epithelium was very flat. The capsule of the neck radiotransmitter implantation site was moderately thick with numerous pieces of hair imbedded in the connective tissue capsule. A fine layer of fibrin had accumulated on the inner surface of the capsule. Small accumulations of macrophages and some focal calcification occurred around the periphery of the capsule. The capsule of the rump radiotransmitter implantation site was thickened due to connective tissue proliferation and fibrinous and fibrinopurulent exudate accumulation on the inner surface. There were also some calcium percipitates, a heavy fibroblastic reaction and granulocytic infiltration in the surrounding adipose tissue. Variable degrees of epidermal hyperplasia and some focal erosion and inflammatory exudate was present in the less affected areas around the incision site. There was full thickness necrosis of the skin over the implant locally.

Bone marrow from a vertebral body has a normal ratio of hematopoietic to fatty marrow with the majority of the cells committed to the granulocytic series. Megakaryocytes were frequent. Mitotic figures were seen occasionally in the granulopoietic clusters. Small lymphocytes or uncommitted stem cells were frequent and often in clusters.

The abscess capsule was thin and the connective tissue poorly organized. There was moderately strong fibroblastic activity, macrophages and suppurative foci in the surrounding adipose tissue.

Foreign body granulomas associated with pieces of hair and what appeared to be porcupine quills, were seen in the submucosa of the external nares. There were small erosions and ulcerations covered with predominately granulocytic exudate and other debris and bacteria in the nasal mucosa around the external nares. There were some edema and plasma cells and granulocytes in the submucosa. In addition to the putrid dried mucopurulent exudate seen at the anterior aspect of the maxilloturbinates, focal necrosis with purulent and fibrinopurulent exudate accumulation was seen in the maxillo- and ethmoturbinate mucosa.

Atelectasis was extensive in all lung samples, and some areas were emphysematous. Large numbers of large foamy cells were seen, either free in some of the alveolar spaces, or lining the alveoli (Figure 7-17). The foamy cell accumulations were most frequent around the larger air passages. The alveolar walls were thickened in some places and an increased number of granulocytes was sequestered in the alveolar capillaries.

The various lymph nodes examined were atrophied to a various degree and lymphoid follicles with germinal centers were absent. The lymph nodal sinuses were markedly congested and contained large numbers of hemosiderin-laden macrophages. Erythrophagocytosis was also very prominent. The spleen was very hemosiderotic and the periarteriolar lymphoid sheaths were small.

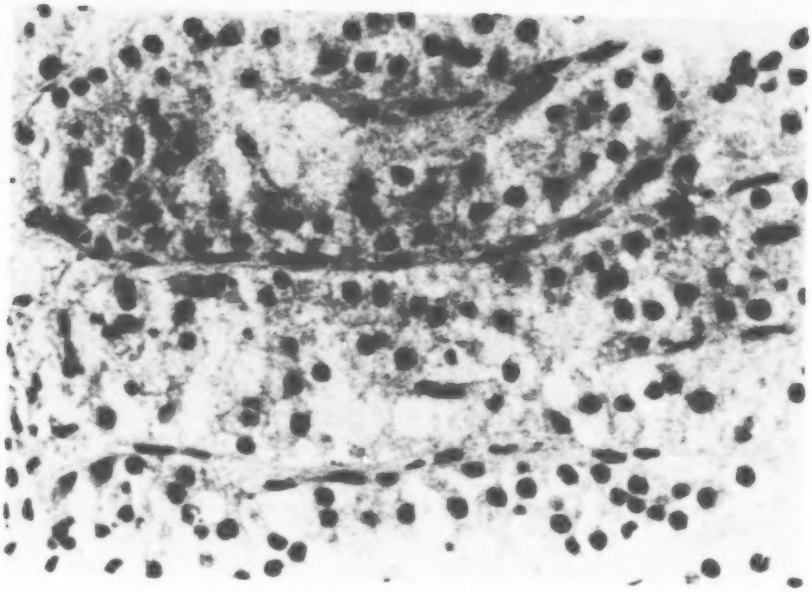

Figure 7-14. Bear 3. Fewer, enlarged, irregularly round and markedly vacuolated cells of zona arcuata. Capsule to the left. Magnification 3750 x.

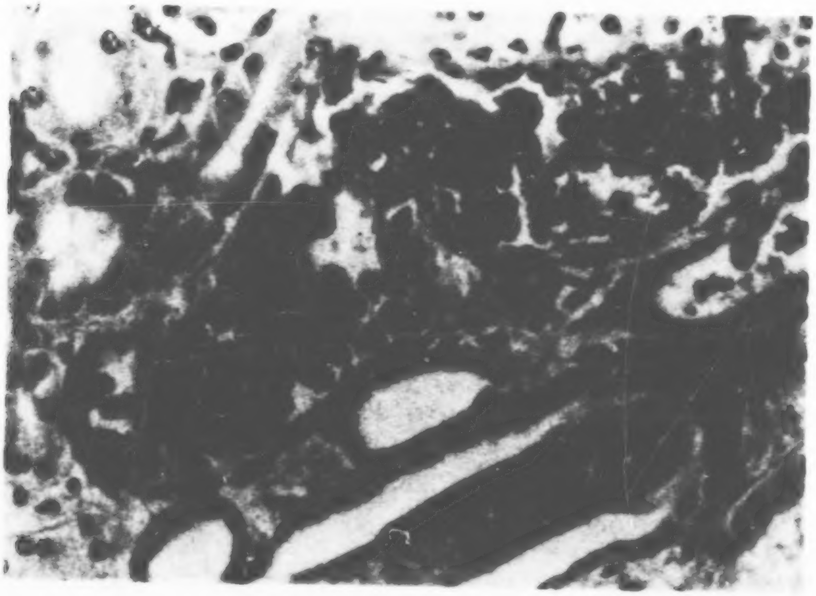

Figure 7-15. Bear 3. Granular hemoglobin cast and sloughed renal tubular cells containing methemoglobin can be seen. Magnification 3750 x.

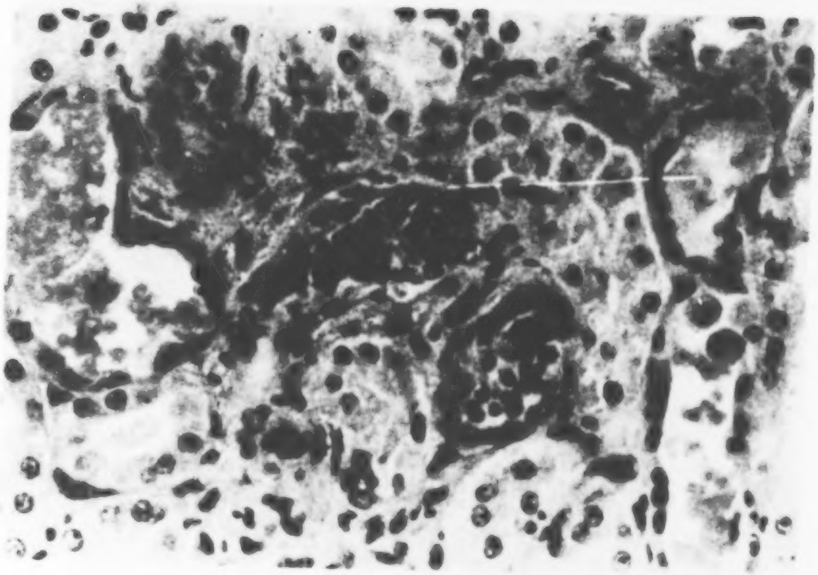

Figure 7-16. Bear 3. Degenerated epithelial cells; some granulocytic exudate is in some of the tubules. Fine calcium precipitates are in the interstitium and the basement membranes. Magnification 3750 x.

Figure 7-17. Bear 3. Large foamy cells are in the alveolar spaces. Magnification 4800 x.

Tongue lesions consisted of localized erosions progressing to deep ulceration with necrosis and calcification of the submucosal connective tissue and the muscle layers. The ulcers were covered with heavy granulocytic exudate, numerous bacteria, and other debris. There was some edema in the lamina propria in the gastric mucosal folds. Erythrocytes and some granulocytes were observed on the mucosa. The duodenal and occasional jejunal crypts were variably distended with dense proteinaceous material and some contained cellular debris. There was some hemosiderin in the lamina propria of the duodenal villi.

The acini of the serous part of the parotid gland varied in size and atrophied acini were frequent. Homogeneous proteinaceous material was seen in some of the ducts. There was a slight to moderate degree of squamous metaplasia of the smaller ducts and degenerated epithelial cells and granulocytic exudate was present in a few of them. The pancreatic acini were small and decreased in size toward the periphery of the lobules. Zymogen granules however, were present in all of them. There was some hepatic cord dissociation and hepatocellular vacuolation of periacinar distribution. The Kupffer's cells were moderately prominent and contained a moderate amount of hemosiderin. There was vacuolation of the epithelium of some of the cholangioles which sometimes contained granulocytic exudate.

7.3.2.3. Bear 4

There was a marked perineuronal vacuolation in areas of the cerebral cortex at the level of the sulci. The Virchow-Robin spaces were also markedly enlarged and there was extravasation of blood around smaller blood vessels. Scattered degenerated and necrotic neurons were seen in the small cell hippocampal pyramidal layer. An occasional sporozoan cyst was seen in the brain parenchyma.

Skin sections from various parts of the body were examined. The dermis formed variably high folds that were covered with variably hyperplastic and acanthotic, hyperkeratotic epidermis. In the thicker areas the epidermal layer was 10 - 12 cells thick. Cornified epithelial cells also distended the hair follicles (Figure 7-18). There was additional epidermal hyperplasia around the incision of the skin temperature radiotransmitter implantation site with accumulation of squamous epithelial cells, inspissated granulocytic exudate, yellowish crystaline material (Furazolidone powder), and other debris. Catgut sutures were present around the incision site. The implant capsule was fairly thick and dense. There was a fair degree of fibroblastic activity in the inner layer. The capsule surrounding the deep body temperature radiotransmitter was less dense, thick and moderately vascularized. No inflammatory cells or other inflammatory exudate was seen.

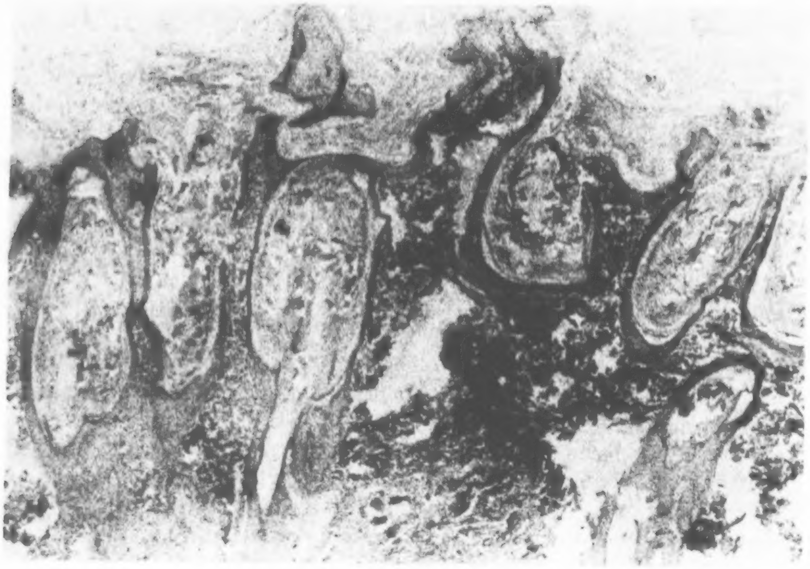

Figure 7-18. Bear 4: Hyperkeratosis and accumulation of cornified epithelial cells in the hair follicles. Magnification 375 x.

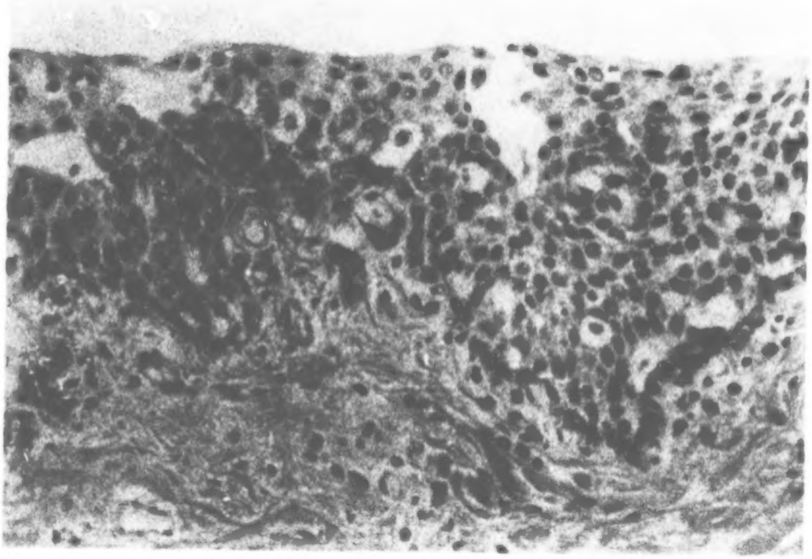

Figure 7-19. Bear 4: Epithelial degeneration and cleft formation with some inflammatory cells in the epithelium and the submucosa of the third eyelid. Magnification 3000 x.

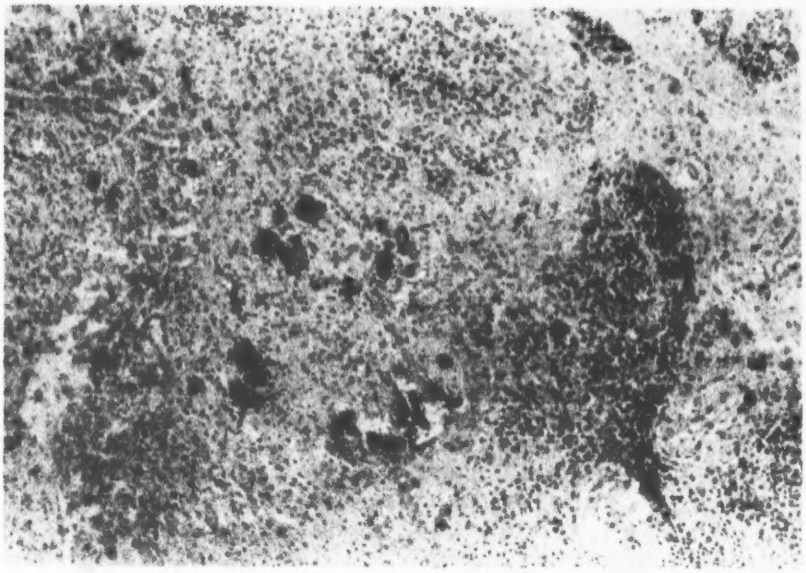

Figure 7-20.

Bear 4. Necrotic area with large bacterial colonies in a peripheral lymph node is demonstrated. Magnification 1200 x.

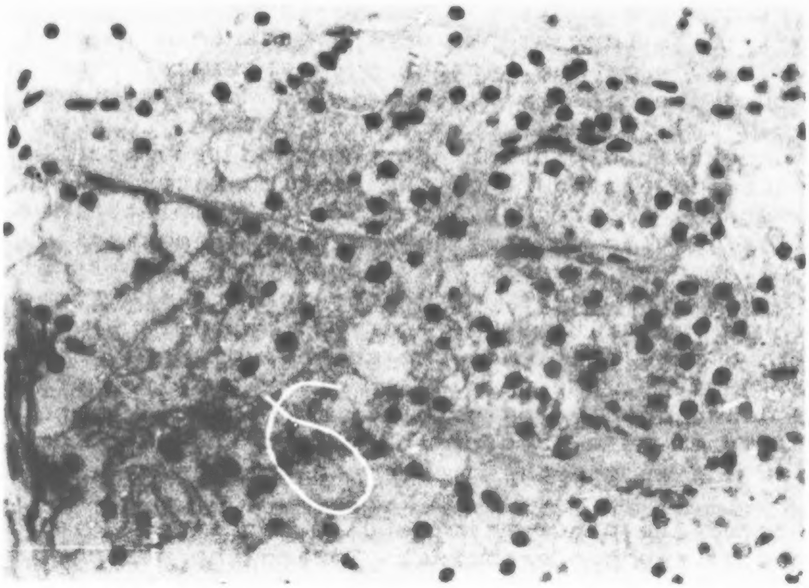

Figure 7-21.

Bear 4. Fewer cells, lack of pallisading pattern and markedly enlarged and vacuolated cells of the zona arcuata can be seen. Capsule to the left. Magnification 3750 x.

The bulbar, palpebral and third eyelid conjunctivae were moderately congested. There was suprabasilary vacuolation, cleft and vesicle formation, and focal cellular degeneration in the thickened conjunctival epithelium which was also infiltrated by a number of granulocytes. In some areas, the cleft formation was transepithelial with fibrin accumulation. Mononuclear leukocytes and plasma cells were seen in small numbers around the conjunctival basement membrane (Figure 7-19). There was a heavy localized accumulation of squamous epithelial cells and other debris on the lingual mucosa and small plasmacytic foci were seen in the submucosa. The folded oesophageal epithelium varied markedly in thickness with the thicker areas predominately at the bottom of the folds. Shallow epithelial erosions were frequent. There was vacuolation and focal granulocytic exudate accumulation in the epithelium and the mucosal surface of the oesophagus. A smaller number of granulocytes was scattered through the epithelial layer and around the basement membrane of the oesophagus and there were large focal accumulations of epithelial cells and other debris associated with numerous fungal elements of morphology consistent with Candida sp. The oesophageal shallow erosions continued into the stomach cardia. The latter cardia erosions were also associated with large number of fungi (probably Candida sp.). A small number of granulocytes infiltrated the mucosa of the cardia. A localized submucosal connective tissue necrosis and a small number of plasma cells was associated with this lesion. There was a small deep ulcer filled with a thick mat of fungal hyphae, of similar appearance to the ones seen in the oesophagus in one of the stomach body sections. The gastric mucosa around the ulcer was moderately hyperplastic and dysplastic. The duodenal crypts were dilated to a variable degree and contained pinkish amorphous to lacy material. Some crypts contained a mixture of the pinkish material and cellular debris. There was a partial to complete loss of epithelial lining in some of the crypts. Hemostasis and hemorrhage was seen in some villi and large numbers of plasma cells were seen in others. The ideal crypts also contained a little pinkish material. The acini of the serous part of the parotid gland were small and the acinar cells had scanty cytoplasm. Some of the acini, and occasionally ducts, contained dense deeply eosinophilic inspissated secretions. A few granulocytes were present in some of the parotid gland duct lumina and around the basement membranes.

The centers of the pancreatic lobules contained acini with a fair number of zymogen granules. Toward the periphery these acini became smaller, less distinct and there was a progressive lack of zymogen granules. The islets of Langerhans were indistinct, the endocrine cells widely separated and in various stages of degeneration and necrosis. A single necrotic lobule with clusters of bacteria was seen in one of the pancreatic sections.

A section through the level of external nares revealed extensive soft tissue necrosis with accumulation of degenerated inflammatory cells, fibrin and other debris. Larger vessels in this area were thrombosed and their walls necrotic. Further from the external nares, but still in the area of cartilagenous nose, the nasal epithelium was vacuolated and contained an occasional degenerated cell and granulocytes. Submucosal edema and thrombosis of the submucosal capillaries were seen in some places. Similar epithelial changes were evident in the larynx.

Atelectasis was extensive in all the lung samples. Small foci containing large foamy cells were scattered throughout the lung sections, alveolar spaces adjacent to smaller air passages, and pleura. A moderate number of macrophages containing dark brown granular pigment, that stained weakly for hemosiderin with the Prussian blue stain, were scattered throughout the lung parenchyma.

The axillary, popliteal, and the retropharyngeal lymph nodes were affected in descending order of severity. The axillary lymph node was extensively hemorrhagic, hemosiderotic and necrotic. Fibrinopurulent inflammatory exudate and large bacterial colonies were present in the peripheral sinus and cortical and medullary areas (Figure 7-20). Similar, but less extensive changes were present in the other lymph nodes. Examination of other lymph nodes, unaffected by the bacterial necrotizing process, revealed sparsely populated cortical areas with lack of lymphoid follicles. The spleen was congested and the periarteriolar lymphoid sheaths were few and small. A few megakaryocytes were seen in the spleen. A bone marrow sample from an unknown location was almost completely fatty marrow.

A large number of renal tubules, at all levels of the kidney, contained hemoglobin casts and brownish hemoglobin degradation pigment. Bilirubin pigment also accumulated as small granules in the renal tubular epithelial cells. Some of the renal tubular epithelial cells were vacuolated, degenerated, and sloughed. A small number of oxalate crystals was present in the renal tubules. Some renal tubules, in addition to the hemoglobin or hemoglobin breakdown products, contained heavy cellular debris. There was some thickening of the basement membranes of the glomerular Bowman's capsules. The seminiferous tubules were non-patent and a few degenerated spermatogonia were seen in the tubules. An occasional degenerated cell was also seen in the epididymal ducts. The hepatocytes varied in size, were vacuolated, and there was hepatic cord dissociation and cellular degeneration in the periacinar areas. Liver changes varied in different lobes. The Kupffer's cells were moderately prominent and contained a moderate amount of hemosiderin. Hepatic erythrophagocytosis was of a moderate degree. Inspissated bile was seen in the bile canaliculi and some of the cholangioles contained toxicappearing granulocytic exudate. The cells of zone fasciculata were large and finely vacuolated. The zona arcuata of the adrenal cortex contained fewer cells and the pallisading pattern was very indistinct or absent. The cells were markedly enlarged, had markedly vacuolated cytoplasm and degenerated cells were seen frequently (Figure 7-21). No clear zone of separation between the zone arcuata and fasciculata was evident. The ratio of zona arcuata to fasciculata was about 1:20. In one of the fat sections, small accumulations of globular, yellowish, fat-insoluble pigment were present in the adipocytes.

7.4. Discussion

7.4.1 Pathological Effects of Oil

It is evident from some of the references (Templeton 1972, Marine Bioassays Workshop Proceedings, 1974, Moore and Dwyer 1974, Meyerhoff 1975, Craddock 1977, Rice *et al*. 1977, Neff *et al*. 1977, Anderson 1979) that considerable information is available on crude oil hydrocarbons effects on lower marine animals including fish.

Oil spills in marine environments have killed or injured thousands of sea birds annually. Lesions reported by Hartung (1966) in these naturally oiled, as well as in experimental birds, were lipid pneumonia, gastrointestinal irritation, fatty livers, adrenocortical hyperplasia, pancreatic atrophy, toxic nephrosis and enlargement of the spleen. It was postulated that at least one major cause of death in the oiled birds was dehydration, Crocker (1974). Geraci and Smith (1976) reported transient eye irritation and minor kidney and possibly liver lesions in experimental seals after immersion in Norman Wells crude oil for 24 hours. Acute mortality occurred, however, when the seals were exposed to the same crude oil by immersion under laboratory conditions. Ingestion studies did not reveal any significant lesions on necropsy within 10 days after ingestion of the oil.

In domestic animals, cattle seem to be most frequently reported as affected by petroleum hydrocarbons ingestion either in the form of crude oil or various distillates. The most important lesions were aspiration pneumonia, enteritis, and acute cystitis, together with degenerative changes in the liver and kidneys. Topically applied kerosene caused hair loss in the horse and severe dermatitis and hematuria in cattle (Blood *et al*. 1979, Smith *et al*. 1972).

Albino rabbits dosed daily with undiluted kerosene for five days until sluggish and stuporous, had hyperemic stomach and intestinal mucosa when autopsied on the fifth day (Gerarde 1959).

A toxicological study in rats, mice and monkeys by Nau et al. (1966), using C_9 - C_{12} petroleum fractions rich in alkylbenzenes and naphtalenes, indanes, cycloparaffins, paraffins, and benzene demonstrated significant changes in bone marrow. Dermal application in mice caused a higher percentage of dermal fibrosis in the benzene treated group. Hyperkeratosis and inflammatory reaction was the more characteristic skin reaction in animals treated with the C_9 - C_{10} and C_{11} - C_{12} fractions. Grossly, the skin was thick, dry and scaly. Other changes seen in the skin were atrophy of the epidermis, perikeratosis and ulceration. Other pathological changes in the internal organs due to C_9 - C_{12} dermal application were focal hemorrhage, pigmentation, inflammatory reaction and atelectasis in the lungs, amyloidosis of the spleen, cortical scarring, sclerosis and necrosis of renal papillae in the kidneys, and necrosis and focal necrosis and amyloidosis in the liver. Dermal fibrosis was the most prominent finding in the skin after dermal application of benzene. Various other changes were seen in other organs in the benzene and n-decane treated animals. Zieserl (1979) in a review article on hydrocarbon ingestion and poisoning in children, lists aspiration pneumonia to be the most common sequel to ingestion of the less viscid petroleum hydrocarbons, and death is almost always due to the pulmonary involvement. Gastrointestinal toxicity has been reported in about 50% of cases. Signs include vomiting, hematemesis and melena. Other less common complications include cardiomegaly, myocarditis, cardiac arryhthmias, hemolysis, albuminuria, glycosuria, hematuria, pyuria, renal cysts, hepatosplenomegaly and liver dysfunction. Hemorrhagic gastritis is commonly associated with uremia in the carnivores (Osborne et al. 1975).

7.4.2. Pathological Findings in Polar Bears

Leukon values remained basically within the normal range in the post-oiling period. When these values are considered in relation to the acute infectious inflammatory lesions - lymph adenitis and abscess formation, and in relation to the leukon response of Bear 2 to a similar infectious inflammatory process in the recovery period from acute renal failure, the leukon circulating compartment (see Chapter 6) did not reflect the severity of the infectious process. Also the bone marrow picture did not indicate a response corresponding to the underlying inflammatory condition. A marked increase in the granulocytic production pool would be expected in the carnivores with such an underlying infectious disease process and a variable response in the storage pool. The bone marrow differential count indicates depression in the marrow mitotic compartment. The lymph nodes were moderately atrophied. The lymph nodal atrophy could be as a result of direct action of some of the crude oil hydrocarbons, most likely benzene, or due to stress-causing elevated adrenocortical hormones and subsequent immunosuppresion. There appears to be elevation of cortisol values in Bear 2 in the later post-oiling period. Cortisol values appear to be within the pre-oiling range in Bears 3 and 4 (see Chapter 6). Control animals or relevant tissue were not available for comparison and thus it could not be commented upon whether there was hypertrophy of the zona fosciculata either related to the captivity and/or oil exposure. The infectious process in Bears 3 and 4 was of an acute to subacute duration and perhaps iatrogenically induced in the post-oiling period by the numerous hypodermic injections. During routine immobilization of polar bears, sterile injections are not achieved under the best of circumstances. Under the experimental conditions, in addition to what would be an expected level of needle contamination, further contamination by crude oil could have initiated an inflammatory reaction of its own, or facilitated introduction of infectious organisms with the hypodermic injections. At any rate it would be expected that a bear with an uncompromised leukon should handle this degree of challenge without any difficulties.

As detailed in Chapter 6, there was a transient elevation of serum GPT, GOT and alkaline phosphatase values indicating some liver damage in Bear 2 in the immediate post-oiling period. Elevation of a whole range of biochemistry values was detected in the serum sample collected from Bear 4 shortly after death. Some of these changes could be correlated with the liver histopathological changes. Others are probably of terminal origin as a result of slow death from dehydration and uremia.

All three oiled bears suffered from acute renal insufficiency that was manifested by markedly elevated serum levels of BUN, creatinine, total protein, absolute and relative serum inorganic phosphorus and absolute and relative decline of serum sodium and chloride. Renal insufficiency in Bear 4 was evident only after examination of the terminal blood sample. The renal failure was of a more gradual onset in Bear 3 and some evidence of inadequate renal function was seen on Day 13 post-oil (P.O.). There was a rapid decline in renal function between Day 22 and 27 post-oil when the animal was euthanized in extremis. Acute renal failure was detected in Bear 2 on Day 34 P.O. The renal function further deteriorated over the next three days and returned to normal over the following 15 days under intensive treatment. Despite considerable quantities of water drunk by all three bears, the animals were unable to maintain positive water balance. It could not be estimated whether fluids and electrolytes were lost through the intestinal tract in diarrhea or through kidneys in polyuria. There is not enough evidence to indicate whether the dehydration was the major cause of renal failure, or if a significant renal lesion was the primary cause. Hemoconcentration preceeded uremia in Bear 2; however, urine of low specific gravity was produced by all bears as could be seen from the examination of a few random samples (see Appendix 5). Sodium was excreted in urine collected around Mar. 20 from Bear 3. The animal was uremic and dehydrated at that time; however, it is possible that the adrenal cortical lesion in the zona arcuata was significant enough to result in aldosterone deficiency and subsequent sodium loss through the kidney. The zona arcuata lesion could be from direct action of the crude oil hydrocarbons or exhaustion phenomenon. The renal microscopic picture was complicated by secondary uremic changes and hemoglobinuric nephrosis. Hemoglobin on its own is nontoxic and renal damage occurs only in association with other etiological factors, Dunnill (1976), such as dehydration and oliguria. The presence of ketones in urine indicates a catabolic state.

Cholesterol serum values were elevated in all three bears. The increase was most likely due to the renal disease since there were no indications of liver damage from the serum leakage enzymes values, and the elevation preceded evidence of renal failure and positively correlated with its severity.

Total bilirubin was very high in the serum sample of Bear 4 at the time of death. This was reflected in the pronounced yellow discoloration of various tissues and also the spinal cord on gross examination. The serum sample obtained was insufficient for further analysis of the bilirubin. The diffuse staining of the brain and spinal cord probably represents an adventitious agonal change and is not an indication of kernicterus.

Markedly elevated CPK and a moderately high cell count correlate well with the brain histopathological changes in Bear 3. A variable degree of epidermal and follicular hyperkeratosis was evident in skin samples from various areas of the body. The changes were most severe in the back and dorsal neck areas. These changes and also the localized alopecia seen in Bear 3, were most likely due to local irritant action of the crude oil hydrocarbons and would correlate well with experimental findings in the laboratory and in some domestic animals. The conjunctival lesion could be due both to hydrocarbon irritation and/or uremia and dehydration. The tongue and oesophageal lesions are consistent with ammonia burn frequently seen in uremia in dogs. Urea excreted in high concentration in the saliva is broken down to ammonia by the oral microflora, and chemical glossitis and oesophagitis ensues. The oesophageal and also the gastric lesions were complicated by secondary fungal infection. It is quite possible that the ingested crude oil also contributed to, or initiated the tongue, oesophageal, gastric and small intestinal changes. Pancreatic atrophy often follows prolonged anorexia and such was the case in the experimental bears. Aspiration of the crude oil into the nasal passages with subsequent irritation of the mucosa could have happened during oiling. Secondary bacterial infection followed on the primary crude oil injury. There was no direct or indirect evidence of crude oil aspiration. The pulmonary gross and histological findings are suggestive of surfactant deficiency. Further identification of the large foamy cells seen in the alveoli was not possible at the light microscopy level. These cells could be either hypertrophied type 2 pneumonocytes or macrophages ingesting the alveolar surfactant. Similar structural changes and decreased level of surfactant were reported by Lykke (1979) and Le Mesurier (1979) in their experimental gasoline inhalation study in rats.

Squamous metaplasia of the salivary duct epithelium could be related to uremia; however, vitamin A deficiency needs also to be considered. It is a well known fact that polar bears accumulate what would be toxic levels in other species of Vitamin A in the livers on their natural diet. The experimental animals were on a diet that most likely was deficient in Vitamin A. Rapid Vitamin A depletion occurs in patients afflicted with a variety of febrile and infectious disease conditions (Moore 1957, Popper et al. 1943) and the same could have occurred in the experimental bears.

7.5. Acknowledgements

I would like to acknowledge the cooperative spirit that prevailed throughout the combined studies on the crude oil effects on polar bears. F.R. Engelhardt, R.J. Hurst and G. Crawshaw were most cooperative. The Veterinary Services Branch personnel in the necropsy area, clinical pathology, the audiovisual section and the secretarial pool were also very helpful. I would like to thank Dr. McPhedran for his support and Mrs. P. Giardino for the very expedient typing of this report.

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APPENDICES

Appendix 1

A MATHEMATICAL MODEL OF HEAT BALANCE IN POLAR BEARS

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August 1980

FOREWORD

Rather than submitting a cryptic list of APL functions, programmes and variables, a first attempt at a more complete description of the mathematical model of heat balance in polar bears is presented here.

The present work emerged partly from the Churchill Polar Bear Project started in 1971 and was supported by the Canadian National Sportsmen's Show, World Wildlife Fund (Canada), the Canadian Wildlife Service, NRC (Can.), NSF (USA), NATO's Research Grants Programme, the universities of Guelph, Ont., and Oslo, Norway, and the Norwegian MAB-Svalbard project.

1. GENERAL INTRODUCTION

The beautiful adaptations of the polar bear to life in the Arctic pose interesting thermal physiological problems. The polar bear has been observed swimming far from the nearest shore and may remain active throughout the year.

It may have been the cumbersome size and temperament of this species that delayed experimental thermal physiological work up to about 1950 (Scholander et al. 1950). Recent work through the former Churchill Polar Bear laboratory has provided quantitative data to a level allowing that a mathematical model of the temperature regulation of this species may now be produced.

Notable progress has been made in constructing models of heat balance in man (Hardy et al. 1970, Bligh 1973). Also some steady state models of the heat balance of both poikilothermic and homeothermic animals are available (Gates 1962, Gates and Schmerl 1975).

In the present work a dynamic model of the temperature regulation of polar bears is described. The present model expands on an earlier model developed for harp seals (Øritsland and Ronald 1978), and concerns primarily dry weather conditions. The model is implemented in APL and compared with in vivo laboratory data from polar bears.

2. BIOLOGICAL PREAMBLE

Regulation of deep body temperatures is a prerequisite for the ability of birds and mammals to stay active under a wide range of weather conditions. The maintenance of a nearly constant deep body temperature is achieved by balancing heat production against heat loss. The balance is controlled by the nervous system: The nervous system monitors peripheral and central temperatures and regulates heat production and loss mechanisms counteracting changes in deep body temperature, although the temperature regulation in homeotherms is automatic cold or heat that is experienced on the conscious level. Hence, the sensation of heat or coldness may result in behaviour aimed at maintaining the thermal balance of the body. Such behaviour may be to change body posture or to move, seeking refuge from exposure.

Thus temperature regulation may be considered under 3 general themes: The automatic controller (the nervous system), the controller action (the regulation of heat production and heat loss) and the controlled system (the temperatures of the body).

Thus is the generality that tropical and arctic mammals have similar deep body temperatures and heat production. The heat loss mechanisms, however, differ sufficiently to compensate for the difference between arctic and tropical climate. Recognizing that heat loss is a product of the temperature difference over an insulating layer and the thermal conductance of the layer, it appears that a significant part of adaptation to heat or cold is a matter of body insulation. Insulation, the inverse value of conductance, depends on the thickness of the insulating layer and the thermal conductivity of the matter of tissue that consitutes the layer. In mammals the three layers: fur, muscle and fat, may be considered significant.

The total heat loss capacity also is determined by the size of the area exposed to the environment. For example, sleeping sled dogs reduce their heat loss beyond what otherwise could be expected from their overall insulation by curling up in 'doughnut' fashion in the cold. Such a body posture serves to minimize the surface area directly exposed to the weather.

In the present work some aspects of our understanding of the temperature regulation of the polar bear is expressed in a mathematical model. The connection between the theoretical model and the anatomy may be indicated by grouping body structures according to their role in a theoretical scheme (Werner 1980):

1) Controller: The brain and the spinal cord.
2) Effectors: Metabolic changes, insulative changes induced by shifts in blood circulation, evaporative changes and thermoregulatory behaviour.
3) Passive system: Muscle, fat, skin and viscera temperature.
4) Receptors: Temperature sensitive nerve structures in the brain, spinal cord, other parts of the body core and in the skin.

3. METHODS

3.1. General model design

The present model is based on the principle (Øritsland 1974) that a total heat balance expression may be constructed by combining the formulas of Keiber and Meeh for heat production and body surface respectively, with the thermal properties of a plane fur sample. The thermal properties of the body, such as the insulative value of the fur, and the energy cost of walking, are derived from experimental data.

The heat production (metabolism) is subject to automatic control in such a way that if the heat generated by walking is not sufficient to maintain deep body temperature the controller will increment the metabolism independent of the walking speed. Also the controller will regulate the heat loss from the body trunk, from the appendages and from evaporation.

The controller operates by comparing the real deep body temperature with "set point" and reference temperatures. Differences or "error signals" between reference and real body temperatures will cause heat production and heat loss to be adjusted in proportion to the error. The adjustments are made towards bringing the body temperature back to the reference value.

3.2. Thermal properties of body structures

3.2.1. Thermal properties of fur.

The thermal insulative value of polar bear fur has been investigated by only a few workers. Scholander *et al*. (1950) reported on polar bear fur insulation values in calm air and water, and Hart (1960) compared polar bear summer and winter furs in air. Also fur insulation in relation to wind speed has been examined (Øritsland and Lavigne 1976, Øritsland 1976). Solar radiation penetrates into polar bear fur thus causing a significant heating at skin level (Øritsland and Ronald 1978). Values of the thermal characteristics of polar bear fur are presented in Table 1. Values on the topographical distribution of fur insulation over the whole body surface have not been reported and the values for the legs thus have to be set arbitrarily.

Table 1. Thermal properties of polar bear fur. The heat transfer coefficient is an equation: $y= a + bV^c$ (W/m^2/°C) where V is the wind speed (m/s). The solar utilization factor SU is defined as the fraction of incident radiation affecting heat loss at the skin level.

a	b	c	SU	Fur Type	Reference
1.2 ± 0.2	-	-	-	?	Scholander et al. 1950
1.2 ± 0.2	-	-	-	winter	Hart 1960
2.1	-	-	-	summer	Hart 1960
3.6	0.05	2	-	summer	Øritsland 1976
-	-	-	0.11	winter	Øritsland and Ronald 1978

3.2.2. Body surface area

The total body surface of mammals may be calculated from Meeh's formula (Hemmingsen 1960). The Meeh constant ranges from 0.08 for seals (Irving 1935, Iversen and Krog 1977) to 0.12 for rats (Richardson). For polar bears, the constants 0.09 and 0.11 were found for the skin and fur surface respectively (Best 1976). The snout represents a fraction of 0.02 and the legs a fraction of 0.41 of the total surface. Somewhat arbitrarily, but also supported by thermography (unpublished) and direct measurements of thermal infrared radiation (Øritsland and Lavigne 1976, Best 1976), a fraction of 0.21 is used in determining the area of the 'thermal legs' in the present model and the fraction (MEF) 0.23 is used for the total appendage area.

3.2.3. Fat thickness and conductivity

The sub skin fat layers on the polar bear are unevenly distributed. On twelve animals between 1 and 25 years, fat layers up to 11 cm thick have been reported (Øritsland 1970). The conductivity of fat is assumed to be 0.205 $W/(m^2 °C)$ (Carlson and Hsieh 1970) in the present model.

3.2.4. Evaporative heat loss

Quantitative measurements of evaporative heat loss has not been performed on polar bears. Best (1976) noted that polar bears will pant. Øritsland (1973) speculated on the basis of thermography that the polar bear may have special adaptations for recondensation of water in expiratory air. Thus in the present work the polar bear is considered as a non-sweating, panting animal. The capacity for respiratory heat loss is in agreement with values for dogs (see Folk 1974) and caribou, set to a fraction (EP) of 0.2 of the total metabolism. The evaporative heat loss fraction may be changed by use of the subroutine NERV.

3.2.5. Temperature responses of the nervous system

'It is widely accepted that the sensors of deep body temperature are concentrated in the hypothalamus area of the brain and that they sense changes in arterial blood temperatures which are of thermoregulatory significance' (Bligh 1973 p. 93). Deep body temperatures of resting polar bears are 36.9°C and increase curvilinearly with activity levels (Øritsland 1969, 1970, Best 1976). The value 36.9°C is used as a reference deep body temperature while separate 'secondary' reference temperatures are used for activating full heat production (TBSM), full heat loss (TBSH) and onset of evaporation (TBEVS). Values for the secondary reference temperatures are taken according to experimental results on humans and other mammals. Thus for the polar bear model full metabolic heat production is established when the deep body temperature (TB) reaches the reference value TBSM = 36.6°C, while evaporative heat loss is started at the same temperature, i.e. TBEVS = 37.2°C (=TBSH), that also produces maximum heat loss due to blood circulation.

Automatic (autonomic) control of the temperature of skin and appendages is well documented for several species of mammals (see Bligh op. cit. and Folk op. cit.). Although skin temperature has been recorded in several polar bears, the actual values triggering automatic defensive action against further reduction have not yet been found. Such defensive action, i.e. increased blood circulation that will keep the skin warm under cold weather conditions, is an important part of the overall heat balance. However, the polar bear may have a 'fish-like' type of collagen allowing skin flexibility at ice water temperatures. Consequently a limiting value of -2°C for the legs is used in the present work. This means that if the environmental conditions would otherwise suppress the leg skin temperature to lower than -2°C, automatic control is activated and -2°C is maintained at the expense of a high heat loss.

3.3. Details of model design

3.3.1. Controller

The reference temperature TBS is simultaneously connected with three other temperatures. These temperatures determine the sensitivity or magnitude of response in heat production, heat loss from the body and the evaporative heat loss. Three major thermal states are possible: "Too cold", "too warm" or "neutral" (Figure A1-1). The state "too cold" implies that deep body temperature is lower than the reference value (TBS). The controller effect is to minimize heat loss and increase the heat production. The heat is dissipated through three parallel routes: One route is represented by the appendages, and the heat loss is regulated by the skin temperature while the fur acts as a permanent insulator against heat loss. On the body trunk both the fur and the fat deposits under the skin act as insulators against heat loss. Thus the body is considered as a double wall, and the insulative value of the inner layer, i.e. the fat, is regulated in a fashion mimicking the effects of changing blood circulation. This is the second route of heat loss. Evaporation, representing the third route of heat loss, is expressed as a simple fraction of the metabolism. The factor (EP) for evaporative heat loss is limited by a maximum value determined from the literature on other non-sweating mammals (Folk 1974).

The controller regulates the heat production by setting the value of a metabolic factor in proportion to the difference between the reference (TBS) and the real (TB) deep body temperature. When the real temperature drops to a lower limit (TBSM) a maximum metabolic factor is created. The rationale for choice of limit values both for heat production and the three routes of heat loss is described under the section for thermal properties of body structures.

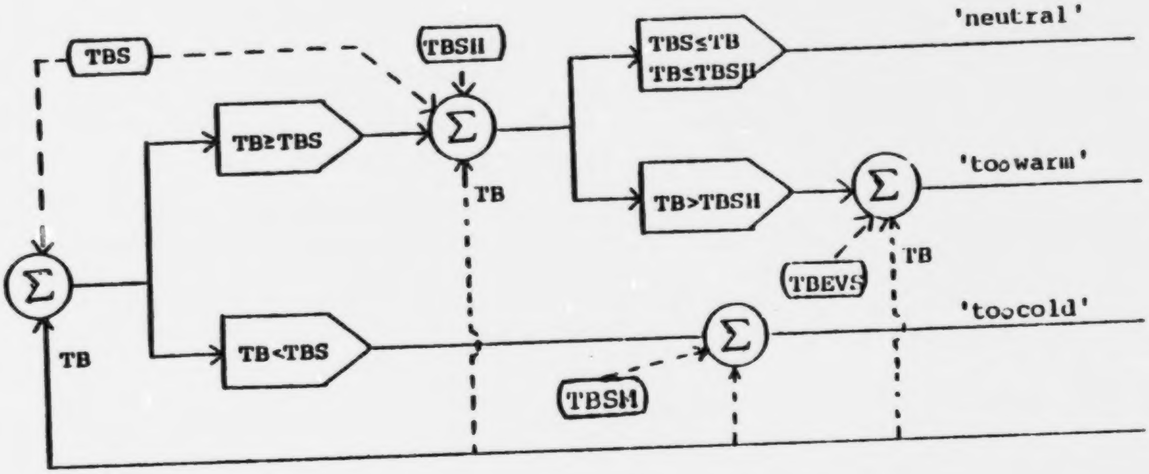
TBS
TBSH
'neutral'
TBS≤TB
TB≤TBSH
TB≥TBS
Σ
TB
'too warm'
TB>TBSH
Σ
TBEVS
TB
Σ
TB
'too cold'
TB<TBS
Σ
TBSM

Figure A1-1. Flow diagram for controller ('thermostat') function in the heat balance model. $\sum$ - comparator that compares by adding input variable values. ▷ - 'gate' that allow only values beyond specified limits to pass for further processing. ▭ - reference or 'quasi reference' temperature values used to trigger or grade responses.

3.3.2 Effectors

The three thermal states, 'too warm', 'too cold' and 'neutral' identified and quantified in terms of temperatures by the controller trigger proportional responses in the heat production and loss mechanisms:

'Too warm' - The state 'too warm' is identified by TB   TBSH and will, when TB reaches the value TBEVS, initiate evaporative heat loss according to the formula:

(eq. 1) $EV = (TB - TBEVS) \times M \times EP$ (W)
where M is the metabolism.

EP is a dimensionless fraction, normally 0.2. The difference TB - TBEVS may maximally have the value 1. Also, under the thermal state 'too warm' and at TB greater than TBEVS, the functional blubber thickness BL and the metabolic factor MF are set to their minimum values, 0.001 and 1, respectively. At the same time the appendage skin temperature TF is regulated to its maximum value: TB-0.5°C. The above actions may be considered as a simulation of maximal circulatory heat loss together with an inhibition of heat production. As mentioned earlier, however, when the animal is walking, the energy, i.e. heat production, due to walking is superimposed on the otherwise minimal heat production.

'Neutral' - For deep body temperatures between the reference value TBS and the upper limit TBSH the model is, from a physiological point of view, in a neutral zone. This implies that the heat production still is at a basal level (MF=1). Appendage skin temperature and body sub skin conductance are reduced, however, to provide thermal balance while evaporative heat loss is zero. The appendage skin temperature (TF) is adjusted according to the formula:

(eq. 2)
$$TF = (-2 + TB)\frac{DTB}{TBSH - TBS} \qquad (^\circ C)$$

where DTB = TBS -TB °C.

The regulation of the functional fat thickness takes place according to:

(eq. 3)
$$BL = 0.001 + TYK\frac{DTB}{TBSH - TBS} \qquad (m)$$

where TYK is the real fat thickness, normally 0.03 m.

The heat transfer coefficient (inverse value of insulation) of the fat is then:

(eq. 4)
$$HCI = KF/BL \qquad (W/m^2C)$$

where KF is the conductivity of fat tissue (0.205 W/m^2Cm).

The actual heat loss from the appendages is:

(eq. 5) $$HLS = (a + bV^c) \cdot (TF - Ta) \cdot AS \qquad (W)$$

where TA is the air temperature (°C)

$a + bV^c$ is the heat transfer coefficient of the appendage fur for wind speed V m/s

AS is the appendage surface area (m^2).

When shifting from rest to walking, an obligatory increment EXR (normally 10 $W/(m^2C)$) of the appendage calm air heat transfer coefficient is made. The rationale for such an increment of appendage heat loss capacity is that the legs move according to walking speed and establish their own wind; i.e., the heat loss regime is to some extent independent of the environmental air movements.

For the body the heat loss is calculated for a double wall:

(eq. 6) $$HLBL = \frac{HLI \cdot (A+B\,V^c) \cdot (TB-Ta)}{HCI + (A+BV^c)} \qquad (W/m^2)$$

where A+BV is the heat transfer coefficient for the body fur.

Solar radiation is set to affect only the body trunk, reducing the heat loss to the final value:

(eq. 7) $$HLB = HLB1 - Su \cdot SR \cdot SFB$$

where SU is the dimensionless solar utilization factor, normally 0.11

SR is the global solar irradiance (W/m^2)
and SFB is the fraction of the body trunk surface considered exposed to the sun.

'Too cold'. The thermal state shifting from 'neutral' to 'too cold' will cause an increment of the heat production by acting on the metabolic factor MF:

(eq. 8) $$MF = (MFMAX-1)\,\frac{TBS - TB}{TBS - TBSM}$$

where MFMAX is the maximum metabolic factor compatible with resting. Normally MFMAX is 2.

The actual heat production is given by the metabolic factor acting on Keibers law:

(eq. 9) $$M = MF(3.4\,W^{0.75})F \qquad (W)$$
where W is the body weight (kg)
and F is a metabolic factor generated by walking:

(eq. 10) $$F = \frac{(0.11 + 0.17\,VLK)\,4.7\,W}{1.162\,(3.4\,W^{0.75})}$$

where (0.11 + 0.17 VLK) is an experimentally derived expression for the oxygen consumption of a 235 kg polar bear during walking (Øritsland and Ronald 1978). The factors 4.7 and 1.162 are conversion factors for oxygen volume to kcal and from kcal/hr to watts respectively. As long as the heat production incremented, which according to the above formulas is not sufficient to establish heat balance (the neutral state), the evaporative heat loss and appendage skin temperature will be kept at minimum values. At the same time the functional fat thickness is at maximum, i.e. equal to the real fat thickness (BL=TYK).

3.4. Discussion of methods

3.4.1. Control theoretical considerations

There are obvious similarities in the performance of animal temperature regulation systems and the systems constructed by engineers. Consequently, analytical methods and concepts from cybernetics and control theory have been applied to thermal physiology. Recent analysis of mammalian temperature regulation has indicated that a neuronal reference signal or 'set point' need not be present in order to obtain the temperature stabilities reported (Bligh 1978, Werner 1980).

Although the term 'set point' is used for TBS in the present model, the controller is more complex than a conventional proportional controller, as appears by its position and action between the temperature triggering full heat production (TBSM) and the temperature triggering full simulation of heat loss due to peripheral vasodilatation (TBSH). The present set point has some of the properties of a null point as described by Bligh (1979). In addition, the present controller has some similarity to a filter by sorting and allowing action under only one or two of three possible thermal states at any time.

It seems possible that the present model/controller may be used for exploring a 'no set point', i.e. a 'null point', hypothesis by replacing TBS with two variables. For example, 'TBSL' and 'TBSMH' could be given values lower and higher than TBS, and used as starting points for the heat loss and heat production effectors respectively. Such a manoeuver would simulate the crossing of two separate heat loss and heat production profiles as discussed by Bligh (1978).

3.4.2. Biological considerations

The present model is unusual in that it calculates heat loss according to formulas valid for plane, not curved, surfaces (Øritsland 1974, Øritsland and Ronald 1978). It has been pointed out earlier, however, that the present approach to heat balance modelling mimicks the effect of curvature

(Øritsland 1978). Thus the characteristics of the seemingly more realistic representation of an animal body by treating it as a cylinder (Porter and Gates 1969, Gates 1975) is retained. The problem of how to simulate postural changes of the body surface (Øritsland 1970) is not handled here or in any other numerically operational heat balance model. Efforts are being made, however, by using a variety of body casts covered with natural fur (Moen).

In the present model the problems of changes in heat dissipation due to changes in body posture is recognized only for the shift from resting to walking. For the resting animal the appendage heat transfer coefficient is kept at a value AAA (typically 3 $W/(Cm^2)$) and the heat loss is limited by regulating the skin temperature between -2°C and half a degree lower than deep body temperature. It could be argued that a concurrent regulation of the appendage fraction of the total body surface can numerically easily be implemented. More knowledge about the real heat transfer coefficient of the appendages should be obtained, however, before such a refinement may comfortably be introduced. It is interesting to note that the heat loss capacity of the legs may be determined indirectly through measurements of the respiratory evaporative heat loss capacity (Øritsland 1980a).

4. RESULTS

4.1. Sensitivity tests

The present model may to some extent be considered an integration of the obvious. Throughout its construction the experimental results have been given priority, and decided the choice of solution if several options are available for specific problems. Sensitivity tests have been part of the methods of construction and only a few have been performed in its present stage of development. It appears that a reduction of fur insulation may be compensated for by an increase in fat thickness, i.e. sub skin insulation. Such a compensation causes a drop in skin temperature, however.

4.2. Comparison with *in vivo* data

4.2.1. Equilibrium body temperatures

The deep body temperature increases during work and in a manner often interpreted as the result of active regulation. For man, the relationship between deep body temperature and the relative work load is nearly linear (see Åstrand and Rodal 1970). For the polar bear, a clearly curvilinear relationship has been found. Polar bears allowed to walk at free will on the treadmill tended to regulate the walking periods in such a way that deep body temperatures were maintained at the same values as found for controlled runs (Best 1976). The values obtained from the living polar bears together with the results of simulations of the model's 'normal' bear are given in Figure A1-2. The model predicts curvilinear relationships as obtained from the in vivo experiments.

4.2.2. Critical air temperature

The concept of critical air temperature is a consequence of Newton's law of cooling as introduced in thermal physiological literature by Scholander and co-workers (1950). According to Newton's law of cooling, an animal subject to decreasing air temperature will decrease its conductance, i.e. increase its insulation until a limit is reached. This point of maximum thermal insulation corresponds to the (lower) critical air temperature. It is worth noting that the term insulation in the context of Newton's law of cooling includes all defense mechanisms against heat loss. Thus the 'Newtonian' conductance term includes insulation achieved by reducing the peripheral blood circulation, increasing the fur thickness by muscular erection of the hairs, reducing evaporative heat loss and curling up in 'doughnut fashion' in order to reduce the heat-dissipating body surface. Experimentally, the critical air temperature is determined for animals resting in respiration chambers. Series of oxygen consumption determinations are made until a linear relationship between the increasing metabolism and decreasing air temperature may be graphed.

Much experimental work has been put into describing the critical temperatures of birds and mammals. Thus although Newton's law of cooling and the critical air temperature derived from it does not predict the thermal energy requirement of animals exposed to natural weather conditions, it may, and perhaps always should, be used as a check point on the performance of more elaborate heat balance models.

Therefore a special subroutine CRITA has been included into the present model. In its present primitive form CRITA will set solar radiation and wind speed to zero and present a graph of metabolic values versus air temperatures as illustrated by Figure A1-3. The extrapolations are done manually.

4.2.3. Climate space

Porter and Gates (1969) suggested that the term 'climate space' be used for the values of air temperature, wind speed and absorbance of solar radiation, limiting the long term heat balance and thus distribution of animals. The calculation of climate space is, according to Porter and Gates, based on a steady state, i.e. not a dynamic heat balance model, and the animal in question is represented by a cylinder with a typical body diameter.

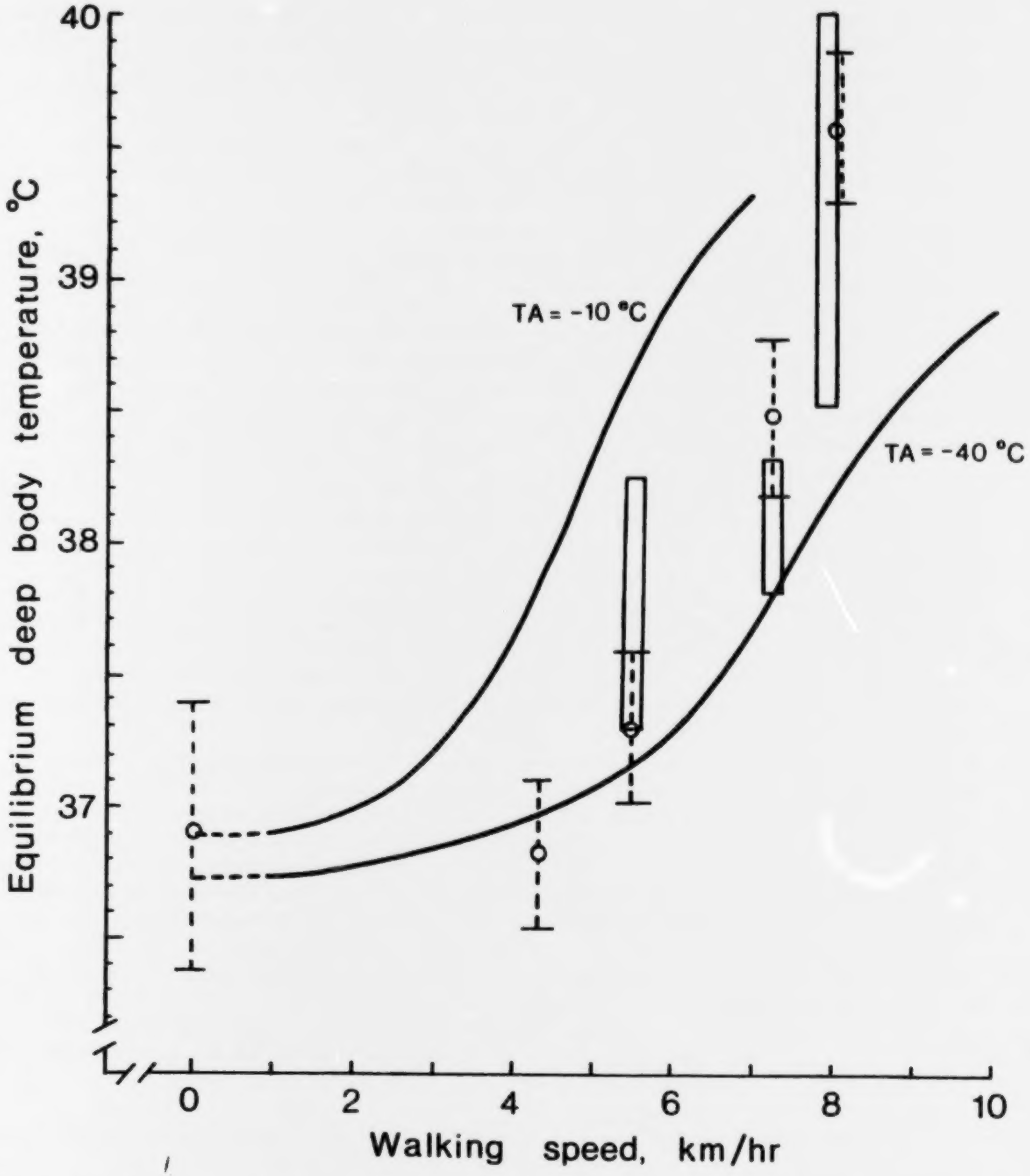

Figure A1-2. Composite drawing of equilibrium deep body temp. as a function of walking speed. Curves illustrate mathematically predicted equilibrium T_b at ambient temps. of -10°C and -40°C. Circles show mean experimental body temp. in controlled treadmill runs. Vertical bars show standard deviation of means. Rectangles show range of T_b when the animal was allowed free access on and off the treadmill, i.e. behavioral temperature regulation.

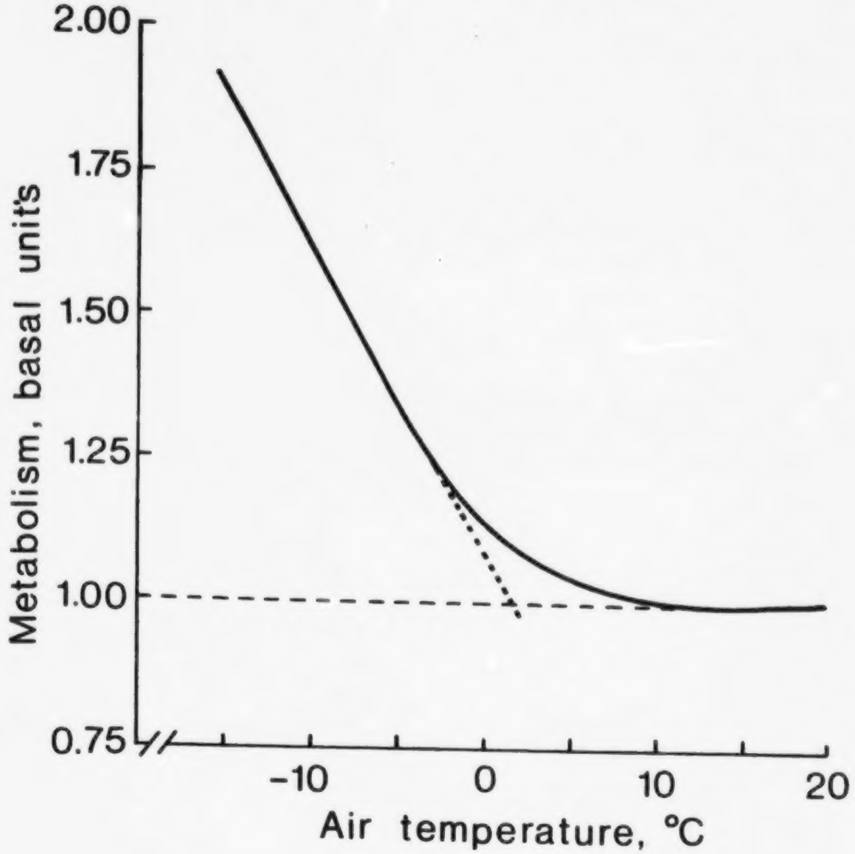

Figure A1-3. Determination of critical air temperature by use of the model. Metabolism is expressed in units of predicted basal metabolism. The critical air temperature is determined by extrapolating (dotted line) the linear part of the metabolism values to intersection with the air temperature at basal level.

Any dynamic heat balance model such as the present one may also be used to determine a climate space by driving the model to its limits of thermal balance. The climate diagrams for a 70 kg and a 200 kg resting polar bear are presented in Figure A1-4. For the determination of the present climate diagrams it is assumed that only a doubling of metabolism is tolerated.

4.2.4. Dynamics of response

In addition to the magnitudes of heat production and loss, the heat capacity of the system will affect the time needed for reaching thermal equilibrium. The specific heat capacity was kept constant at 3100 J/(kg C) for the present simulations. The time needed for reaching thermal equilibrium from a starting body temperature of 36.9°C in a normal polar bear subject to three different regimes of activity level and ambient conditions is presented in Figure A1-5. An aspect of how equilibration time may depend on ambient cold stress, other variables being constant, is illustrated in Figure A1-6.

5. PROGRAMME

The subroutine details of the APL computer model and related references are available on request. Please write Dr. N.A. Øritsland, Institute for Zoology, University of Oslo, Oslo, Norway.

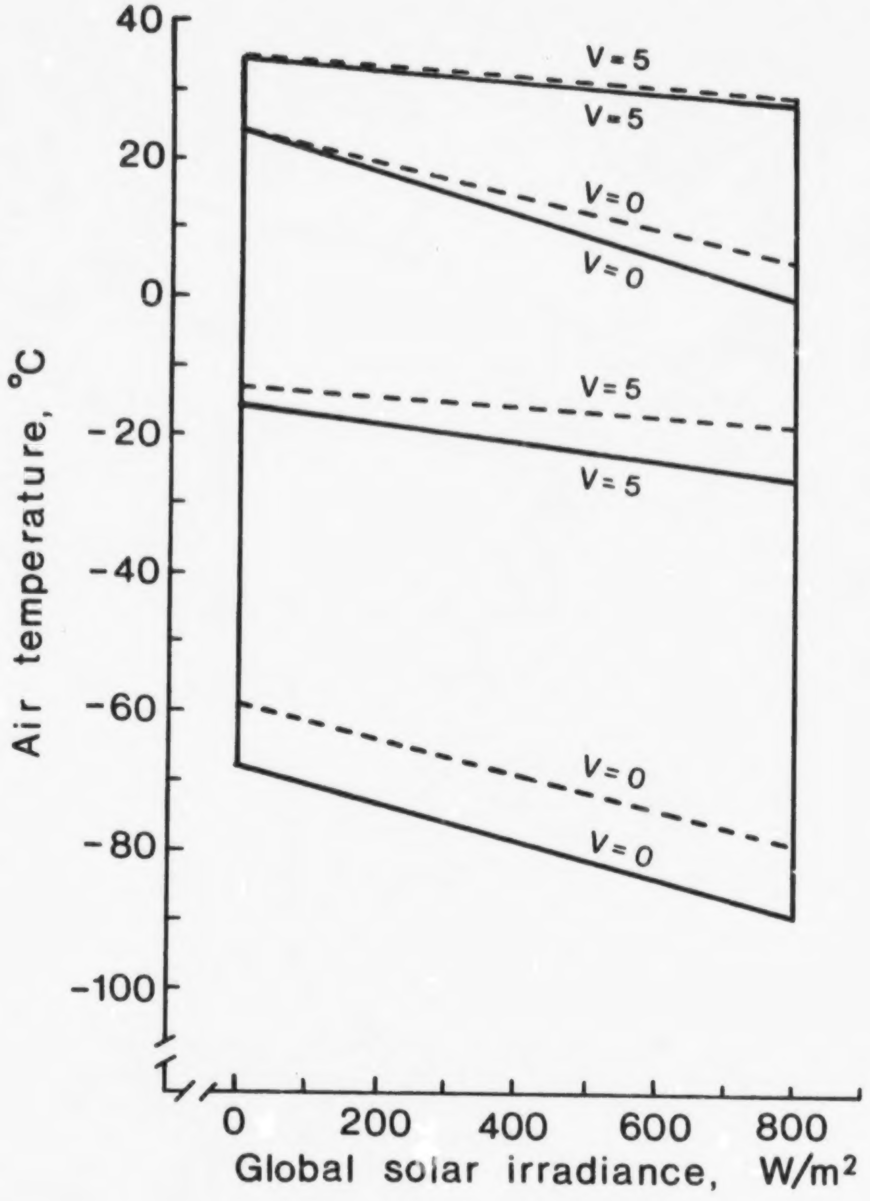

Figure A1-4. Climate diagram for a 200 kg (——) and a 70 kg (- - -) polar bear exposed to wind speeds (V) of 0 and 5 m/s. Limits of tolerance are calculated for a resting animal under the assumption that only a doubling of basal metabolism can be tolerated. Subcutaneous fat thickness is 3 cm. and the fur insulation is set to $1.2 + 0.05\ V^2$ and $3 + 12\ V^{0.1}$ W/(m²°C) for body and legs respectively. The solar utilization coefficient is 0.11.

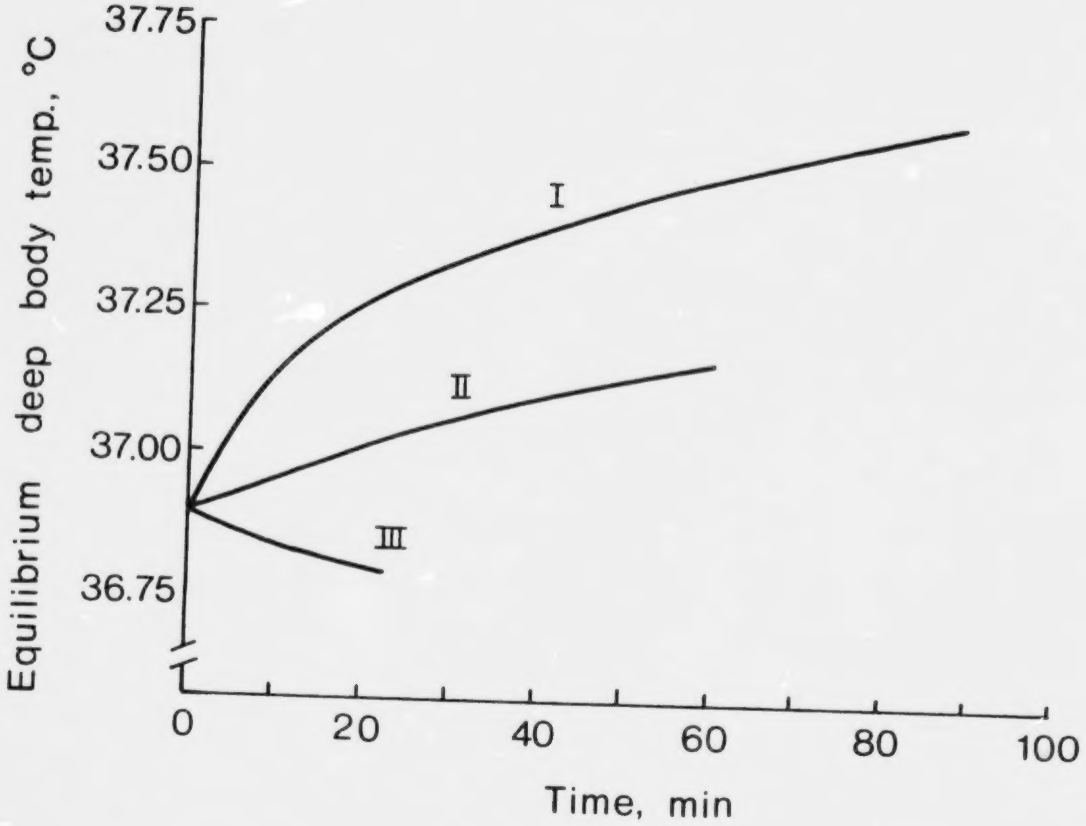

Figure A1-5. The establishment of stable deep body temperatures, as predicted by the model, illustrated for a 'normal' polar bear in calm air and not exposed to solar radiation. The end point of the lines represent equilibrium deep body temperature. The energy balance regimes simulated are:
I walking speed 5 km/hr at air temp. -20°C
II resting at air temp. 0°C
III resting at air temp. -40°C.

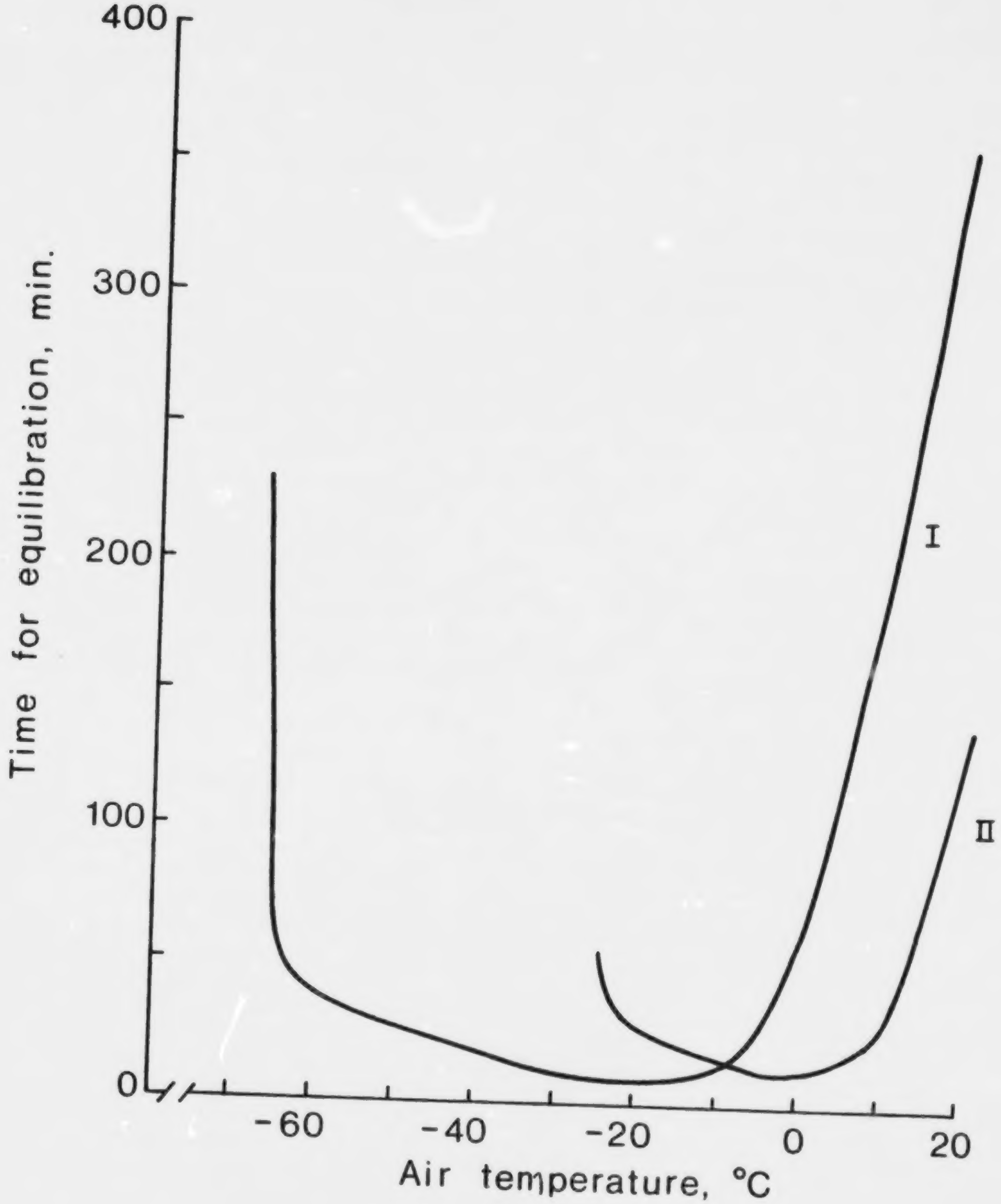

Figure A1-6. The time needed to reach stable deep body temperature (T_b) from an initial T_b of 36.9°C, expressed as a function of air temperature. Wind speed and solar irradiation are zero, and the maximum metabolism is set at 2 times basal. Line I depicts a normal bear and line II depicts a 70 kg animal with a thin fat layer (1 cm) and half the body fur insulation value of the normal bear.

Appendix 2

CLEANING OIL-FOULED FUR

R.J. Hurst

1. Introduction

Attempts to physically remove crude oil from various wildlife species have met with qualified success (Williams 1978, Williams *et al*. 1978), largely due to the stress of handling combined with the ill effects of the cleaning solvent itself. Since there was little precedent with fur bearing mammals, and none with polar bears, cleaning techniques were tested on fur samples a month prior to live animal exposure.

Solvents specifically manufactured for oil chemical breakdown (i.e. "Shell-Sol 50") were not tested for three reasons: a) the potential harsh effects on the thermal and water repellent properties of the fur, b) the potential toxic effects to the live animal itself, and c) the lack of ready availability for the treatment of contaminated polar bears in an arctic environment. Instead, sawdust in combination with commercially available shampoos, detergents and cleaning solvents was tested.

2. Pelt Samples

2.1. Methods

A section of raw polar bear hide was cut into 10 cm squares and soaked in a container of sea water covered with a 1 cm slick of Midale crude oil. Each section was agitated with rapid hand movements for five minutes to simulate the swimming motion of a live animal. It was then allowed to remain in the solution for another 25 min. After 24 hrs. at ambient laboratory temperatures (-20°C), a series of cleaning techniques and solutions were applied to separate squares of fur. Eight solvents were investigated. Factors affecting the suitability of any product and thus tested, were: amount of solution, concentration of solution and temperature of rinse solution. A photo-record was made and a decision made on the protocol to follow when cleaning the live experimental animals.

2.2. Results

The best results were obtained by washing the fur in a 15 % solution of Sunlight dish soap (Lever Brothers, Toronto, Canada), and then rinsing the fur in warm (30°C) water. Sawdust served to physically absorb oil and to absorb water on completion of the cleaning process.

3. Live animals

3.1. Methods

Each of the three polar bears used in the oil study was cleaned 17 days after exposure to oil. A cleaning room was prepared and equipped with an abundant amount of sawdust, 30 liters of 15 % "Sunlight" soap solution, and a 750 liter (200 gallon) metal rinse container. This tank was filled with warm (30°C) water. The polar bear was immobilized and wheeled to the cleaning room, at which time a protective cloth was fastened over its eyes. Three persons were necessary in the cleaning procedure. Vital signs of each animal were closely monitored and additional injections of immobilizing drug administered when necessary.

The first stage involved repeatedly working sawdust into the fur and brushing out the oil-sawdust mixture. The soap solution was then applied and mixed as a lather to the skin level over the entire body. Warm water was poured over the fur removing most of the soap, and the bear was then lowered and immersed in the rinse tank. After 15 minutes, the bear was raised out of the tank, and water was removed from the fur by generous use of sawdust and brushing. At this time the animal was returned to a holding cage containing a sawdust "bed".

3.2. Results

To the observer's eye, most of the oil was removed by the described procedure and the animals appeared clean on completion. Oil at the skin level was difficult to reach, and as a result, was not entirely removed. In the following days or weeks a small amount of oil seemed to "work its way" out to the fur surface. The entire process, to be successful, took several hours (3.5 hrs. average) and thus required the administration of 2-3 times the "normal" dosage of immobilizing drug. The water seemed to stimulate nervous co-ordination in the bears and thus extra care in monitoring the level of immobilization was necessary.

4. Discussion

The described cleaning process was visibly successful in removing oil from polar bear fur; however, several qualifications are necessary. The actual amount of oil remaining on the fur and the detrimental effects of such an amount have not been quantified. Since chemical immobilization normally presents some risk and the amount of drug necessary to adequately clean a polar bear exceeds normal dosage, the effect of drugging should be considered in relation to the advantages of cleaning.

Before cleaning could be performed in an actual oil spill situation, considerable preparation must be made in respect to facilities, materials and personnel. Finally, until such time as the external coating versus ingestion aspects of oil have been elucidated, the potential advantages of a polar bear cleaning contingency plan should be carefully evaluated relative to the logistical and monetary expenses inherent in such a plan.

Appendix 3

SCHEDULES OF EXPERIMENTATION

BEAR 2

October 09: - captured by Manitoba Govt., Female, 337 lbs (143.9 kg), quiet animal.

09-28 - used in attractants study (U. of Montana).

29 - Blood. - Bear moved into treadmill to upgrade cage.

31 - Moved back into re-designed cage.

December 10 - Blood. - Area around femoral shaved for future blood extraction.

February 02 - operation - transmitters 13 (T_b); 05 (T_s)-

02-07 - jab stick streptopen antibiotic in recovery cage (22 ml i.e. 3.6 ml/day for 6 days).

09 - 25 lbs. meat.

11 - to respiration chamber for first measurement of experiment.

12 - 2nd respiration measurement.

13 - 3rd

14 - 4th Total measurements = 4.

15 - moved to treadmill, Blood - OK, 15 lbs of meat, no run on treadmill.

16 - run at 1330 - no O_2; at 1635 - no O_2.

17 - 1100 - complete; 1400 - complete.

18 - 1130 - complete; 1930 - complete Total runs=8.

19 - 1100 - complete; 1520 - complete (with O_2) =6 + 1 run at 2.0 m/sec.

moved to wind chamber; 12 lbs meat.

20 - Behavior and temp. measurements begun at 1400 hrs. continued as control until Feb. 21: 1400 hrs. 10 hrs wind; 14 hrs no wind.

February 21 1400 hrs - moved toward pool

1442 - in pool until 1457 (15 minutes)

1515 - in recovery cage

1533 - begins licking

1900-2200 - fans on; wind. 2200-0200 hrs - no wind.

22 - 0200-06 - wind - continuous - 4 hrs on, 4 hrs off until Feb. 23: 1000 hrs.

Total time = 24 hrs control; 10 hrs wind
43 hrs experim.; 19 hrs wind

23 - behaviour finished at 1000 hrs;

1200 hrs - drugged and moved to respiration chamber; blood, no respiration measurement.

24 - 2400 hrs measurement - OK.

25 - 2330 hrs measurement - OK Total measurements =2.

26 - moved into treadmill; blood - OK; given 13 lbs of meat; 1830 - first treadmill attempted; treadmill cold; - first time engine does not work.

27 - treadmill will not work in morning; 2110 - run is OK.

28 - work on treadmill with no success; bear moved to a cage.

Feb 29-Mar 03 - moved to sawdust cage, uses sawdust, consumes much snow, 15 lbs of meat.

March 03 - treadmill run 1700, 2100 OK

04 - treadmill run, 1300 OK; 9 lbs of seal
Total runs = 4.

05-08 - in cage with sawdust, water (vs snow) given for first time, thirst appears insatiable, reasonably alert but will not eat.

March 09 - Washing - 3 hour procedure, legs and shoulders most covered with oil, some oil near skin in these places but otherwise OK. Total drug, twice normal dose in 5 injections, blood attempt unsuccessful - moved into sawdust box for warmth.

BEAR 3

October 17 - captured in culvert trap by Manitoba Govt., male, sub-adult.

- Blood. Weight 363 lbs (164 kg).

31 - Blood OK. (only whole blood sample).

December 10 - Blood OK. Area around femoral shaved for future blood extractions.

February 09 - 25 lbs of meat.

15 - Operation - transmitters: T_b 15, T_s (neck) 01, T_2 (rump) 04.

15-20 - given jab stick of antibiotic streptopen - 19.8 ml (3.3 ml/day) for 6 days while in recovery cage.

20 - moved into respiration chamber, 18 lbs of meat, measurement at 2300 hrs.

21 - measurement at 2400 hrs.

22 - measurement at 2400 hrs.
Total measurements = 3 (2 are OK).

23 - moved into treadmill, blood -OK; weight = 163 kg., 18 lbs of meat, run at 2230 - no O_2.

24 - run at 1645 and run at 2020.

25 - run at 1200 - no O_2 - run at 1600 (OK).
Total runs = 5
(with good O_2) = 3.

26 - behaviour begun at 1400 in recovery cage, no wind. - Wind at four hour intervals until Feb. 27th at 1730.

27 - 1730 - moves to pool.
1754 - into pool until 1847 (53 minutes).
1852 - back into recovery cage, begins licking fur.
1900-2200 - no wind, 2200-0200 wind, alternating every 4 hrs until Feb. 29: 1000 hrs.
Total time = Control 2730 hrs,
Wind = 12 hrs.
Experim. = 39 hrs.
Wind = 20 hrs.

February 29 - moved into respiratory chamber, blood - OK, weight = 406 lbs (184 kg), measurement at 2345. Possibly unrecovered from drug.

March 01 - measurement at 2300.

02 - measurement at 2330.

03 - measurement at 2400.
Total measurements = 4
(reliable measurements) = 3

04 - moved into treadmill, blood - OK. Weight = refuses to eat seal or other.

05 - does not respond well to treadmill, lethargic; treadmill not functioning properly, runs cancelled.

06 - moved into recovery cage for observation, continues for 24 hrs. - given water for first time, thirst appears insatiable.

07 - refuses to leave recovery cage, will not walk, rarely stands.

09-10 - behaviour subdued but reasonable, consumes much water.

11 - moved into sawdust cage - definitely oily animal.
- responds to sawdust well.
- drugged for blood - difficult.
- weight = 330 lbs (150 kg).

12-14 - quiet, using sawdust, will respond aggressively.

15 - cleaned - total time = 4 hrs, total drug = Ket. 2425 mg., Rom. 2025 mg.
- long washing process - heavily oiled, much near skin - washing was sucessful however with little oil left.
- fur molt, great hunks on the mid back extending to sides pulls out in handfulls; however leaves clean guardhairs in regular but thinner pattern. Appears only undercoat shed.
- moved back into sawdust cage with sawdust replaced
- weight = 313 lbs (before washing).

BEAR 4

November 03 - first captured by Manitoba Govt. Painted 16.

07 - recaptured by Man. Govt.

13 - captured in culvert trap - moved to laboratory. Blood.

16 - Blood - OK. Note: apparently has an unusual tolerance to drugs.

December 10 - Blood - Weight loss (144 to 129 kg).

February 08 - Operation - transmitters T_b 10, T_s 03, weight 122 kg.

08-13 - Jab stick - given 24.3 ml streptopen in 6 days (4.05 ml/day).

14 - 25 lbs of meat.

16 - moved into respiration chamber, weight = 115 kg, measurement at 2300 hrs.

17 - measurement at 2300 hrs.

Total measurements=4.

18 - measurement at 2300 hrs.

19 - measurement at 2400 hrs.

20 - moved to treadmill, blood OK, 15 lbs of meat, measurement at 1950 - no O_2.

21 - measurement at 1745 and at 2010.
22 - measurement at 1245. Total runs = 4
- 12 lbs of meat (with O_2) = 3.

23 - moved into recovery cage, observation begun at 1400 hrs, no wind for 4 hrs, wind 4 hrs etc. until Feb. 24 at 1400 hrs.

24 - 1400 hrs towards pool, in pool ar 1419-1449 (30 min.)
- 1454 back into recovery cage, no wind to 1900, wind to 2300, no wind to 0200, wind to 0600 and every 4 hrs change to Feb. 26, 1000 hrs.
- given snow ad libitum for duration in recovery room.

Total time = Control 24 hrs,
Wind 12 hrs,
Experim. 40 hrs,
Wind 20 hrs.

February 26 - moved to respiration chamber, blood OK, weight = 138 kg. Measurement at 0030 hrs.

27 - measurement at 0045 hrs.

Total measurements=3.

28 - measurement at 2330 hrs.

29 - moved to treadmill, blood OK, weight = 114 kg, animal does not appear well, treadmill runs cancelled.

March 02 - moved into sawdust cage - very listless, no response or interest in food, curls up in sawdust, ate volumes of snow.

02-07 - no interest in seal or any other food, craves snow.

07 - feces still dark - high oil content (apparently), given water, appears insatiable.

08 - still no appetite, more H_2O with vitamin supplements, a little more active i.e. will usually rise on provocation, seen pounding on bars for few min.

09 - seems OK, but very quiet, making full use of sawdust box.

10 - moved out of sawdust cage and into new adjacent cage, a little uncoordinated, but walked on own.

11 - listless.

12 - Washed - total time = 3.5 hrs. total drug = Ket. 1650, Rom = 1650 mg. Blood OK. Washing went well, little left at skin, moved back into new cage without sawdust.

Appendix 4

TRANSMITTER IMPLANTATION

P.D. Watts

1. Introduction

Operating procedures used with all three oil-study polar bears follow the methods as outlined by Watts (unpublished). A brief description is given here and is followed by a more detailed general outline of animal care and surgical procedures.

2. Surgical procedure - Oil Project

Transmitters were stored in a 5 % sterilizing solution of savlon for at least 24 hours prior to surgery. In surgical preparation, each implantation site was scrubbed for 10 minutes with a solution of iodine-based disinfectant (Betadine Surgiscrub, Purdue Frederick Co., Toronto). Absolute elimination of the possibility of hair contamination had to be compromised so as not to jeopardize the validity of the temperature measurements, i.e. a small area was shaved.

The animal was then moved to the operating room and the site encircled with sterile surgical drapes. For deep body transmitter implantation, a primary incision 6 cm. long was made in the skin. Underlying adipose tissue, fascia and abdominal musculature were dissected and the transmitter imbedded in the rectus abdominus close to the peritoneum. A series of sutures (0 and 00 dexon) were applied to close the fascia and finally, the primary skin incision. Hemostasis was maintained by the application of sterile gauze and pressure. Implantation of skin transmitters was less complicated. A small incision (4 cm.) was made in the skin. A subcutaneous pocket was hollowed out by blunt dissection and the transmitter fitted into this space. A series of Dexon sutures closed the skin incision.

Post surgical care included spraying the site with 'Topazone' (Austin Lab., Guelph, Ontario) and sealing it with a clear spray, 'Aeroplast' (Parke Davis and Co., Brockville, Ontario). An intramuscular injection of 4 cc of antibiotic 'Streptopen' (procainepenicillin G. and dihydrostreptomycin, Allen and Hanburys, Toronto, Ontario) followed each operation. Sterile 'ophthalmic' ointment (Wellcome Vet. Div. Burroughs Wellcome Ltd., Lasalle, Québec) was applied to the eyes to minimize drying and irritation. Injection of streptopen and the application of opthalmic ointment became standard procedure whenever a bear was immobilized.

Following the operation, each animal was held in a clean cage for a 5 day observation period. The small cage size facilitated jab stick injection of 4 cc streptopen/day for the five day period.

3. Physiological transmitter implantation.
(Planned surgery and the management of soft tissue damage)

3.1. Introduction

Quick healing of soft tissue damage is partially dependent upon the nutritional state of the study animal and is adversely affected by dehydration. For this reason pre-and post-operative treatment will include a twice daily check for available, clean moisture intake material (as compared to a once a day check during general holding), and the best and most naturally nutritious food source available. Generally, pre- and post-operative treatment will each consist of one week. Tissue damage will be avoided, and tissue trauma minimized. This will help to forward the healing process by providing the maximum situation for the formulation of granulation tissue and fibroplasia. Every precaution will be taken to ensure that the wounds are mechanically and bacteriologically clean. Blood clots will be avoided as they form an excellent media in which to propagate bacteria. This will be done by utilizing good hemostasis.

Blunt dissection will be used whenever possible in order to obtain surgical goals.

3.2. Preparation and Incision

To minimize damage resulting from cold exposure and maximize the reliability of the scientific measurements, the hair shave will be minimized. Cut hair will be controlled by the use of a small vacuum cleaner onsite in the prep room. Wound site scrub will consist of a ten minute scrub with surgical soap and sterile gauze pads. This will be carried out in the standard way with all action away from the incision site. For each incision a single drape with an operating window will be employed (Leonard 1968) as the surgery is not complicated enough to warrant multiple drapes. It may be possible to supplement the single drape with disposable drapes that stick to fur.

Each drape will be held in place by four backhaus clamps. The primary incision will be done with a sterile, unused blade that is discarded after the skin incision is complete.

3.3. Suturing

It is important to make a firm approximation of all tissues. Sutures are placed far enough from the incision so as not to interfere with healing and not tied so tightly as to strangulate the tissue. Internal stitches should always be minimized. Apposition of tissue from suturing is promoted whenever possible, by following the natural stress lines of the tissue when making the original incision. Suture material will be cut rather than broken, when using the round or mayo needles, and the needle will then be forced over the suturing material. The low tensile strength of fat makes the approximation of tissue impossible; however, dead spaces should be avoided. Skin sutures are approximately 5 mm apart. The first closure line is interrupted using a subcutaneous incision. The second closure line is interrupted subcuticularly if tensile strength is high enough, if not, subcutaneously. Sutures are a little farther (1-3 mm) from the incision than normal to allow the use of a surgeon's knot (two wraps in the first throw, one wrap in the second), which has excellent holding power and helps to compensate for the reduced possibility of observing the stitches post-operatively.

3.4. Laparotomy

Some special considerations must be given for the implantation of the deep body temperature transmitters, as it requires a deeper intrusion into the study animal's body than the skin transmitter implant or the heart rate transmitter implant. The tensile strength of the fascia is twice that of the skin and forty times that of the muscle, thus it plays an important role in preventing wound breakdown. A cranial midline incision is employed, that minimizes wound stress and helps to promote maximum tissue approximation.

The fascia is dissected 1-2 mm to the side of the aponeurosia. Blunt dissection is carried out in the external oblique, internal oblique (not readily recognizable), transversus abdominus, (not readily recognizable) and the rectus abdominis muscles. The transmitter is placed close to the peritoneum embedded in the rectus adbominis.

3.5. Ligation

Under normal procedure ligation is not necessary. If necessary, primary ligation is by hemostat or forceps, then tied off with a nontransfixed square knot, For larger vessels the ligation is transfixed and then the ligature is passed around the entire vessel.

3.6. Postoperative Care

Post-operative care begins with an aerosol coating of topazone and is followed by an aerosol plastic bandage, aeroplast. Antibiotics are administered daily for five days by jabstick while the study animal is in the recovery cage.

4. Reference

Leonard, E.P., 1968.
Fundamentals of small animal surgery. Saunders Co., Toronto. 265 p.

Appendix 5

MISCELLANEOUS HOLDING DATA

(Summarized by F.A. Juck, Based on: 1) Holding Records for Polar Bears at Churchill, Manitoba, compiled by Øritsland *et al*. (see Chapters 3 and 4, and 2) Urinalyses by F.A. Juck).

TABLE A5-1

IMMOBILIZATION SUMMARY - BEAR # 1

DATE	DRUG(S) DOSAGE (mg)	METHOD OF INJECTION	ROUTE	INJECTION SITE	TIME TO EFFECT (min)	IMMOBILIZATION TIME (min)	PURPOSE
Nov. 02/79	*Ket. HCl 360 **Xyl. HCl 360	jab stick	I.M.	neck	5	78	Blood collection
Dec. 10	Ket. HCl 450 Xyl. HCl 450 & 200	"	"	"	7	80	Blood collection
Mar. 09/80	Ket. HCl 1075 Xyl. HCl 825	"	"	shoulder	no effect	not achieved	Intended surgery
Mar. 10	Ket. HCl 1250 Xyl. HCl 1250	"	"	rump	no effect	not achieved	Intended surgery
Mar. 25	Ket. HCl 600 Xyl. HCl 600	"	"		5		Transport to Winnipeg Assiniboine Zoo

*Ketamine hydrochloride
**Xylazine hydrochloride

TABLE A5-2

IMMOBILIZATION SUMMARY - BEAR # 2

DATE	DRUG(S) DOSAGE (mg)	METHOD OF INJECTION	ROUTE	INJECTION SITE	TIME TO EFFECT (min)	IMMOBILIZATION TIME (min)	PURPOSE
Oct. 09/79	Phencyclidine HCl 150 Promazine HCl 150	jab stick	I.M.	-	17	-	Capture and transfer from snare.
28	*Ket. HCl 750 **Xyl. HCl 350	"	"	shoulder	4	100	Transfer within the lab. and blood collection.
31	Ket. HCl 190 Xyl. HCl 190	"	"	neck	7	54	Transfer within the lab.
Dec. 10	Ket. HCl 850 Xyl. HCl 850	"	"	"	6	120	Blood collection
Feb. 02/80	Ket. HCl 820 & 200 Xyl. HCl 820 & 300	"	"	"	21	230	Surgery
11	Ket. HCl 530 Xyl. HCl 680	"	"	"	18	in den	Placed in respiratory chamber
15	Ket. HCL 710 Xyl. HCl 800	"	"	"	25	130	Moved to treadmill; blood collection
23	Ket. HCl 720 Xyl. HCl 720	"	"	rump	20	in den	Placed in respiratory chamber; blood collection
26	Ket. HCl 730 Xyl. HCl 500	"	"	neck	10	148	Moved to treadmill
Mar. 09	Ket. HCl 800 & 900 Xyl. HCl 800 & 700	"	"	"	20	69	Washing for three hours
11	Ket. HCl 800 Xyl. HCl 800	"	"	shoulder	23	73	Blood collection
15	Ket. HCl 800 Xyl. HCl 400	"	"	rump	18	88	Blood collection
25	Ket. HCl 760 Xyl. HCl 760	"	"	neck	5		Transportation to Winnipeg

*Ketamine hydrochloride
**Xylazine hydrochloride

TABLE A5-3

IMMOBILIZATION SUMMARY - BEAR # 3

DATE	DRUG(S) DOSAGE (mg)	METHOD OF INJECTION	ROUTE	INJECTION SITE	TIME TO EFFECT (min)	IMMOBILIZATION TIME (min)	PURPOSE
Oct. 17/79	Phencyclidine HCl 180 Promazine HCl 150	jab stick	I.M.	shoulder & neck	6	70	Transfer to L-9 lab. and blood collection
Oct. 31	*Ket. HCl 720 **Xyl. HCl 720	"	"	back leg	6	72	Blood collection
Nov. 16	Ket. HCl 300 & 100 Xyl. HCl 200 & 100	"	"	neck	8	90	Blood collection
Dec. 10	Ket. HCl 850 Xyl. HCl 850	"	"	neck	8	90	Blood collection
Feb. 15/80	Ket. HCl 900 Xyl. HCl 900	"	"	shoulder	12	120	Surgery-radio transmitter implantation
20	Ket. HCl 900 Xyl. HCl 900	"	"	rump	20	not recorded	Moved to respiratory chamber
23	Ket. HCl 700 Xyl. HCl 700	"	"	neck	30	120	Moved into treadmill; blood collection
29	Ket. HCl 875 Xyl. HCl 525	"	"	rump	24	60	Moved into respiratory chamber; blood collection
Mar. 04	Ket. HCl 850 Xyl. HCl 425	"	"	neck	10	100	Moved into treadmill; blood collection
11	Ket. HCl 850 Xyl. HCl 850	"	"	rump	11	not recorded	Moved to saw dust cage; blood collection;
15	Ket. HCl 825 & 1600 Xyl. HCl 825 & 1200	"	"	neck	5	80	Four-hour washing.
25	Ket. HCl (Not known)				- not known -		

*Ketamine hydrochloride
**Xylazine hydrochloride

TABLE A5-4

IMMOBILIZATION SUMMARY - BEAR # 4

DATE	DRUG(S) DOSAGE (mg)	METHOD OF INJECTION	ROUTE	INJECTION SITE	TIME TO EFFECT (min)	IMMOBILIZATION TIME (min)	PURPOSE
Nov. 13/79	Phencyclidine HCl 200 Promazine HCl 150	jab stick	I.M.	neck	13+	60	Transfer to L-9 Lab.
16	*Ket. HCl 300, 100 & 200 **Xyl. HCL 300, 100 & 200	"	"	"	6	100+	Blood collection
Dec. 10	Ket. HCl 800 Xyl. HCl 800	"	"	"	10	120	Blood collection
Feb. 08/80	Ket. HCl 750 & 300 Xyl. HCl 750 & 300	"	"	neck rump	7	83+	Surgery - radio transmitter implants
16	Ket. HCl 650 & 200 Xyl. HCl 675 & 200	"	"	neck	20	90	Moved to respiratory chamber
20	Ket. HCl 650 Xyl. HCl 650	"	"	"	17	-	Moved to treadmill; blood collection
26	Ket. HCl 625 Xyl. HCl 310	"	"	"	12	-	Moved to respiratory chamber; blood collection
29	Ket. HCl 600 Xyl. HCl 300	"	"	"	7	80	Moved to treadmill; blood collection
Mar. 12	Ket. HCl 600 & 1050 Xyl. HCl 600 & 1050	"	"	shoulder	5	60	Washing and blood collection

*Ketamine hydrochloride

**Xylazine hydrochloride

TABLE A5-5

FOOD CONSUMPTION - BEAR # 1

DATE		AMOUNT (kg)	DESCRIPTION OF FOOD	COMMENT
Aug.	05/79	2.3	meat scraps	
	07	1.8	"	
	08	2.3	"	
	09	1	"	
	11	2.3	"	
	12	1	"	
	13	2.3	"	
	15	2.7	"	
	17	2.7	"	
	19	2.7	"	
	22	2.7	"	
	28	6.8	"	
Sept.	08	6.8	"	
	13	9.0	"	
	19	5	"	
	25	7.3	"	
Oct.	03	6.8	"	
	10	6.4	meat scraps - 50 % fat & meat	
	20	6.8	"	
	27	6.8	"	
Nov.	03	6.8	"	
	10	6.8	"	
	17	6.8	"	
	24	6.8	"	
Dec.	01	6.8	"	
	08	6.8	"	
	19	4.5	seal	
	27	4.5	seal	
Jan.	06/80	9	meat scraps - 50 % fat & meat	
	13	6.8	"	
	21	6.8	"	
	28	6.8	"	
Feb.	03	3.6	"	
	09	11.4	meat scraps - 80 % fat	
	15	5.9	"	
	21	6.8	"	
	28	4.5	"	
Mar.	03	4.1	seal (meat and blubber)	
	08	5.5	beef - 60 % fat	
	12	1	seal	
	15	1	"	
	16	6.8	beef - 50 % fat	
	18	4.5	"	
	20	2.7	"	
	22	2.7	"	
	23	2.7	"	
	24	1.8	"	

TABLE A5-6

FOOD CONSUMPTION - *BEAR # 2

DATE		AMOUNT (kg)	DESCRIPTION OF FOOD	COMMENT
Oct.	10/79	not known	seal	
	20	6.8	meat scraps, 50 % fat & meat	
	27	6.8	" "	
Nov.	03	6.8	" "	
	10	6.8	" "	
	17	6.8	" "	
	24	6.8	" "	
Dec.	01	6.8	" "	
	08	6.8	" "	
	19	6.8	seal	
	27	6.8	seal	
Jan.	06/80	9.0	meat scraps, 50 % meat & 50 % fat	
	13	6.8	" "	
	21	6.8	" "	
	28	6.8	" "	
Feb.	09	11.3	meat scraps (mostly fat - 80 %?)	
	15	5.5	meat scraps 90 % fat)	
	20	5.5	" "	
	26	5.9	" "	
Mar.	02	6.8	" "	
	03		seal	Offered but not eaten

*Oiled Feb. 21

TABLE A5-7

FOOD CONSUMPTION - *BEAR # 3

DATE		AMOUNT (kg)	DESCRIPTION OF FOOD	COMMENT
Oct.	20/79	6.8	meat scraps, 50 % fat & meat	
	27	6.8	" "	
Nov.	03	6.8	" "	
	10	6.8	" "	
	17	6.8	" "	
	24	6.8	" "	
Dec.	01	6.8	" "	
	08	6.8	" "	
	19	6.8	seal	
	27	6.8	seal	
Jan.	06/80	9.0	" "	
	13	6.8	" "	
	21	6.8	" "	
	28	6.8	" "	
Feb.	03	3.6	" "	
	09	11.4	meat scraps, 80 % fat	
	20	8.2	" "	
	23	8.2	" "	
Mar.	03	4.1	seal (meat and blubber)	did not eat the food
	06		seal	did not show any interest
	08	1.0	beef	no interest

*Oiled Feb. 27

TABLE A5-8

FOOD CONSUMPTION - *BEAR # 4

DATE		AMOUNT (kg)	DESCRIPTION OF FOOD	COMMENT
Nov.	17/79	6.8	meat scraps, 50 % fat & meat	
	24	6.8	" "	
Dec.	01	6.8	" "	
	08	6.8	" "	
	19	6.8	seal	
	27	9.0	seal	
Jan.	06/80	9.0	meat scraps, 50 % fat & meat	
	13	9.0	" "	
	21	11.3	" "	
	28	9.0	" "	
Feb.	03	3.6	" "	
	14	11.3	meat scraps, 80 % fat & 20 % meat	
	20	6.8	" "	
	24			Bear was oiled on this date and refused any food afterwards

*Oiled Feb. 24

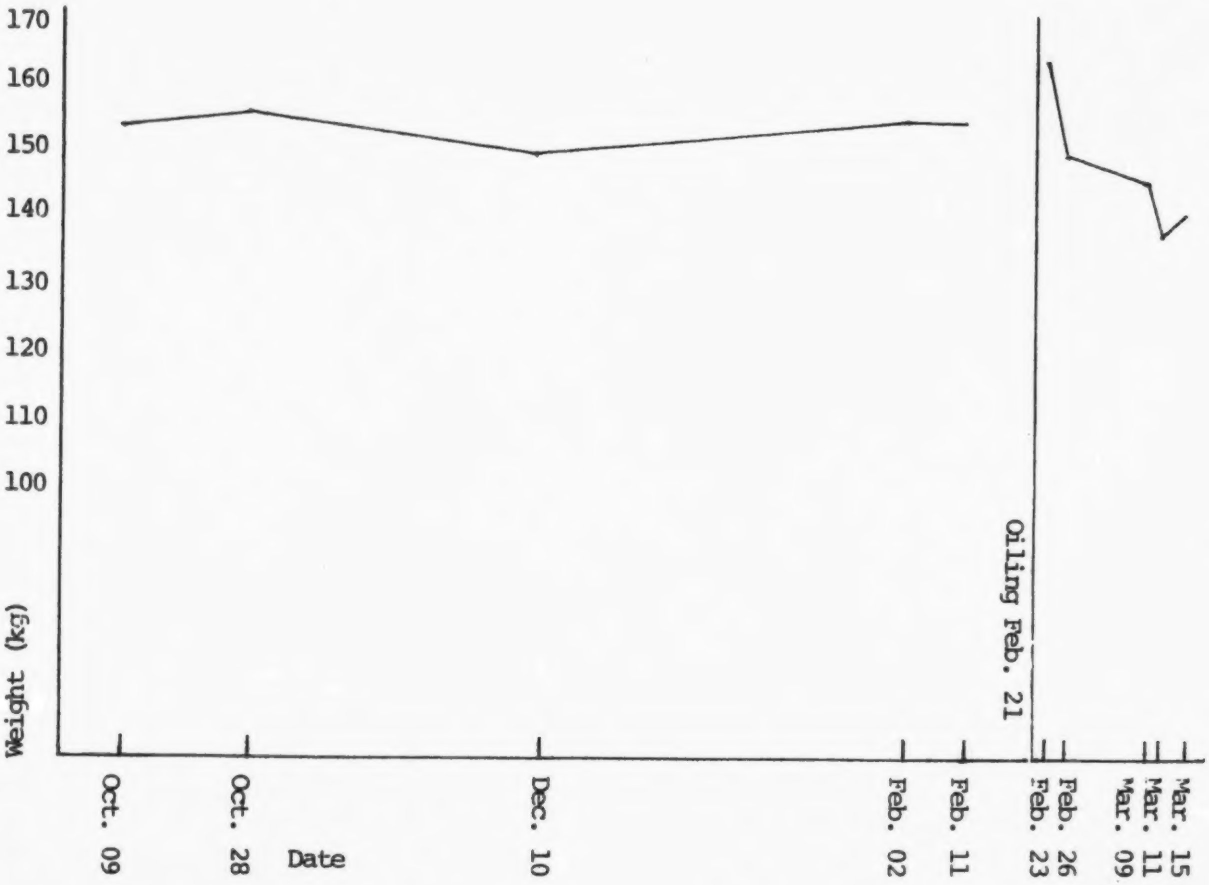

Figure A5-1.
Body Weight - Bear # 2

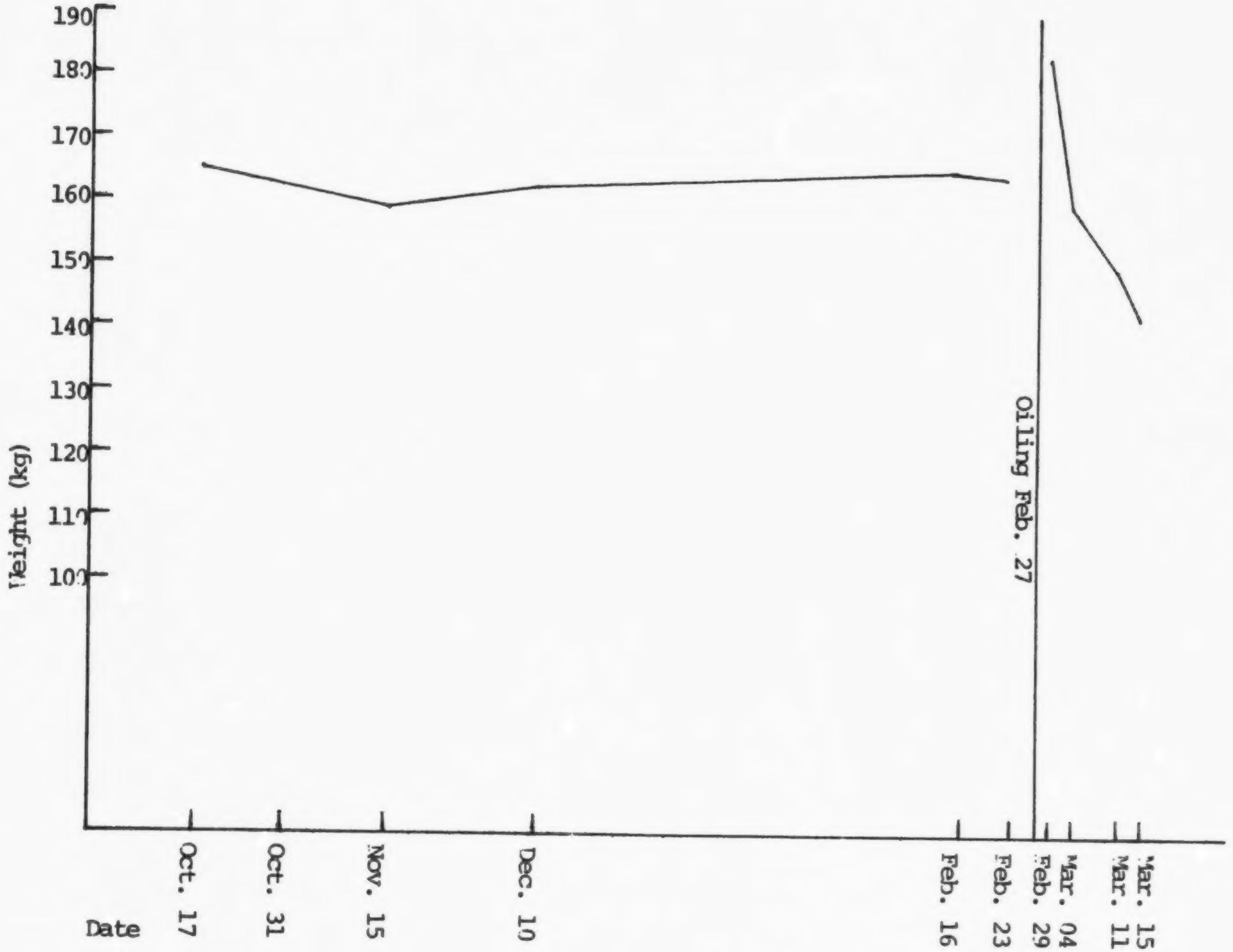

Figure A5-2
Body Weight - Bear # 3

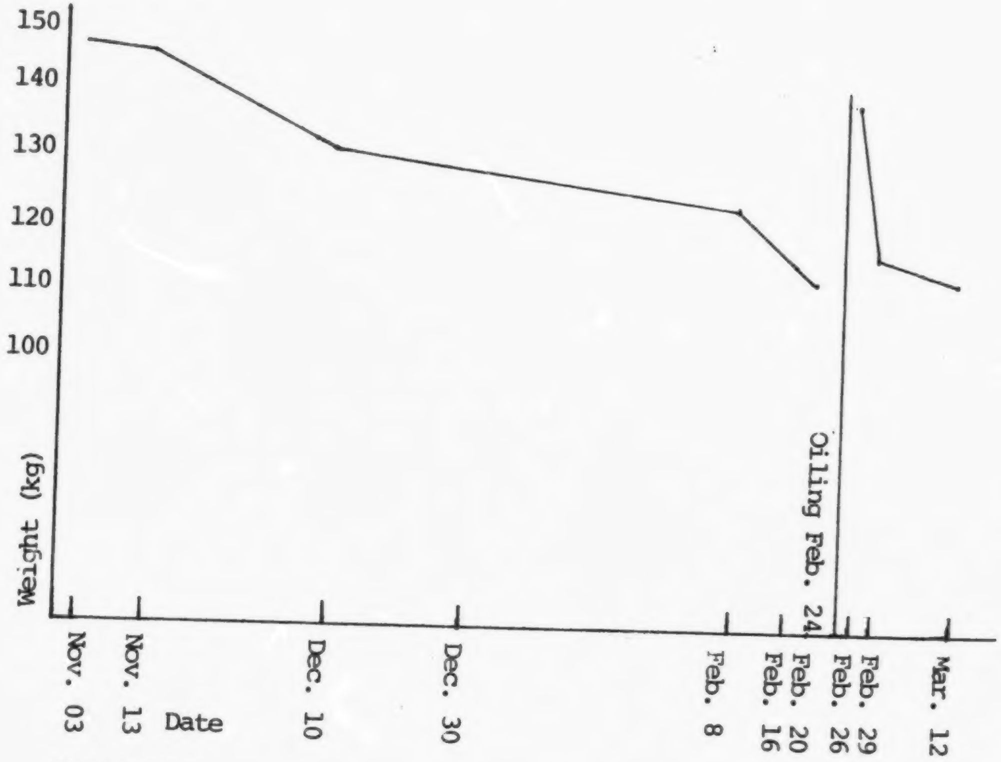

Figure A5-3.
Body Weight - Bear # 4

TABLE A5-9

WATER INTAKE - *BEAR # 2

DATE	AMOUNT (liters)	SA-37 SUPPLEMENT (grams)	OTHER
Jan. 18 - Feb. 10	snow	-	-
Feb. 11 - Feb. 14	none	(animal in respiration chamber)	
Feb. 15 - Feb. 19	snow	-	-
Feb. 20 - Feb. 25	none	(animal in wind chamber)	
Feb. 26 - Mar. 07	snow		
Mar. 08	4 water	7	
09	3.0	5	
10	2.5	4	
11	5.7	8	
12	9.5	16	
13	9.5	8	
14	9.5	21	
15	7.5	8	
16	8.0	16	
17	5	7	
18	no records available		
19	no records available		
20	no records available		
21	9.0	22	44 grams powdered egg
22	8.5	23	45 "
23	8.0	22	45 "
24	10	26	55 "

*Oiled Feb. 21

TABLE A5-10

WATER INTAKE - *BEAR # 3

DATE	AMOUNT (liters)	SA-37 SUPPLEMENT (grams)	OTHER
Jan. 18 - Mar. 07	snow ad libitum		
Mar. 08	4	7	
09	3	5	
10	2.5	4	
11	3.8	5	
12	9	16	
13	9	19	
14	12	22	
15	4	8	
16	13	22	
17	11	19	
18 - 20	records not available		
21	9	22	44 grams powdered egg in water
22	7	17	34 "
23	4	10	20 "
24	1	10	20 "

*Oiled Feb. 27

TABLE A5-11

WATER INTAKE - *BEAR # 4

DATE	AMOUNT (liters)	SA-37 SUPPLEMENT (grams)	OTHER
Nov. 17, 1979	snow ad libitum		
Mar. 07, 1980			
08	4.0	7.0	
09	3.0	5.0	
10	2.5	4.0	
11	13.0	24.0	
12	2.0	8.0	
13	9.0	19.0	
14	10.0	22.0	
15	2.0	5.0	
16	5.0	8.0	
17	1.5	4.0	
18 & 19	2.0	not known	not known
20	none accepted		
21	none accepted		

*Oiled Feb. 24

TABLE A5-12

URINALYSIS - BEAR 1

PARAMETERS	DATES
	March 31/80
Colour	dark
Turbidity	4+
Sp. gravity	1.015
pH	9.0
Protein	30 mg %
Glucose	neg.
Ketones	neg.
Blood	2+
Bilirubin	neg.
Urobilinogen	
WBC	
RBC	few
Other	
Sodium (m Eq/l)	22
Potassium (m Eq/l)	30

TABLE A5-13

URINALYSIS - *BEAR 2

PARAMETERS	DATES					
	March 15	March 16	March 17	March 18	March 25	March 27
Colour					medium	dark
Turbidity					4+	4+
Sp. gravity	1.024	1.023	1.021	1.020	1.017	1.021
pH	6	5.5	6	7	5.5	8.0
Protein	30 mg %	trace	trace	trace	30 mg %	trace
Glucose	neg.	neg.	neg.	neg.	neg.	neg.
Ketones	2+	2+	1+	1+	3+	1+
Blood	2+	neg.	neg.	1+	slight	slight
Bilirubin	neg.	neg.	neg.	neg.	neg.	neg.
Urobilinogen		neg.	neg.	neg.		neg.
WBC					occasional	
RBC						
Other					1.	1. & 2.
Sodium (m Eq/l)						
Potassium (m Eq/l)						

*oiled February 21

1. - slight amorphous debris
2. - loaded with bacteria

TABLE A5-14

URINALYSIS - *BEAR 3

PARAMETERS	DATES	
	both samples collected around March 20	
Colour	medium	light
Turbidity	2-3+	2-3+
Sp. gravity	1.020	1.016
pH	5.0	5.5
Protein	30 mg %	30 mg %
Glucose	neg.	neg.
Ketones	2+	2+
Blood	trace	trace
Bilirubin	neg.	neg.
Urobilinogen		
WBC		
RBC	2-5/HPF	2-3/HPF
Other	mod. amorph. debris	slight amorph. debris
Sodium (m Eq/l)		30
Potassium (m Eq/l)		15

*oiled February 27

TABLE A5-15

URINALYSIS - *BEAR # 4

PARAMETERS	DATES
	March 15/80
Colour	
Turbidity	
Sp. gravity	1.020
pH	5.5
Protein	trace
Glucose	neg.
Ketones	2+
Blood	neg.
Bilirubin	
Urobilinogen	neg.
WBC	
RBC	
Other	
Sodium (m Eq/l)	
Potassium (m Eq/l)	

*oiled February 24

Appendix 6

MEDICAL HISTORY AND TREATMENT AT ASSINIBOINE PARK ZOO: POLAR BEAR 2

G. Crawshaw

The bear arrived at the Assiniboine Park Zoo on the evening of March 25th, 1980. She had been immersed 33 days previously and had not eaten for 20 days. She was exceptionally docile and would not react to human presence except when stimulated. The bear remained in the crate overnight and was anaesthetised the following morning.

Physical examination revealed that the bear was extremely thin, dehydrated and anaemic. The coat was in poor condition, matted and contained oil but showed no tendency to depilate. The bear was lethargic but there was no evidence of neurological disorder. Temperature, pulse and respiration were normal. Urine, which was passed in small quantities, was dark, specific gravity 1.012, and contained blood and little protein. Blood samples were taken, which confirmed the presence of anaemia and gave evidence of renal failure with associated serum electrolyte abnormalities and some liver dysfunction.

Treatment consisted of injections of glucose and electrolyte solutions intravenously and subcutaneously, antibiotics, vitamins and an anabolic agent. A transmitter was surgically removed from the jugular groove. Anaesthesia was maintained for 90 minutes and recovery was uneventful. That day the bear drank a large quantity of water and some milk.

The following day (27/3/80) the bear was livelier, more alert and more aggressive. A variety of foods were offered (horse meat, fat, dog chow, carrots, bread, fish) but none were accepted. Following the addition of salt, water intake increased to about one gallon per day and urine production also increased.

On the 29th of March the bear began to deteriorate and had still not eaten. In addition to previous food, she was offered ground fish, margarine, fruit and vegetables. She was further depressed the next day, would lie down most of the time and was very unresponsive. Lameness in the left foreleg was noticed. She was again anaesthetised for examination and treatment. Hydration had improved but the mucous membranes were pale grey. No evidence of oral ulceration or uraemic breath was detected. Haematological and biochemical tests indicated the uraemia had increased and there was severe erythrocyte and leukocyte depression. Treatment was similar to the previous occasion with intravenous and subcutaneous fluids and electrolytes. A stomach tube was passed, and a large quantity of bile-stained water and dark tenacious bile removed. The stomach was flushed several times with physiological saline and filled with 50 % glucose solution and a gastric antacid. No abnormalities were found in the left leg. On this occasion, exceptionally high doses of drug were required to maintain anaesthesia and recovery was rapid.

The bear appeared worse on 31/03/80 and no food had been eaten. Treatment was repeated on 1/4/80. Only a small quantity of bile was obtained from the stomach which was then filled with a mixture of glucose and cereal gruel. Laboratory results were essentially the same but with some reduction in the uraemia. She was again anaesthetised on 4/4/80 and, in addition to previous therapy, was washed in warm water and detergent. Lab data showed there had been an improvement in the renal function and later that day she ate a few fish.

During the next three days the bear's appetite and activity improved. In addition to fish she ate horse meat, liver, heart and dog chow.

On 7/4/80 an abscess broke on the bear's right shoulder and an oral antibiotic (amoxicillin) was prescribed pending results of bacterial cultures. Two days later she showed some hind limb weakness and by 12/4/80 she had become extremely weak on her rear legs. A potassium supplement was provided. Her appetite remained moderate at approximately 2 lbs. fat, 6-10 lbs. smelt and a little dog chow each day. Left foreleg lameness recurred on 13/4/80 and the following day, though stronger in her hind quarters, she would not put the leg to the ground. Various enteric organisms had been cultured and the antibiotic was changed.

She was anaesthetised on 14/4/80. Two suppurating wounds were present on the lateral aspect of the right foreleg and two on the nape of the neck. The area of the left elbow was extremely swollen and oedematous but no suppurative point was found. Another early septic site was detected on the posterior aspect of the right hing leg. Cultures were again made from the abscesses which were cleansed and the bear again received fluids subcutaneously. A stomach tube was passed but only a small quantity of gastric fluid obtained.

Blood analysis revealed considerable improvement in renal function and serum electrolyte levels, as well as evidence of red cell regeneration. Blood and pus continued to drain from the neck and right shoulder for several days and on 16/4/80 the left elbow area broke, producing a large quantity of pus. Following culture of Pseudomonas and Proteus sp., the antibiotic was again changed to carbenicillin intramuscularly four times daily.

During the following week two abscesses on the right hind leg and one on the left hind leg broke open, but the bear continued to eat well and appeared unaffected. There were then no further developments for several weeks, her appetite increased to 4 lbs. dog chow, 5 lbs. fat, 1 lb. carrots, daily and she gained considerable weight. However, she continued to drink large quantities of water.

On 25/5/80 another abscess broke on the hip region and the following day one developed on the bear's nose. These all produced large quantities of pus and blood before healing gradually. The bear was anaesthetised and transferred to an outdoor pen on 29/5/80. Besides the nasal abscesses no other sores were found. The coat was heavily matted but healthy hair was visible close to the skin. The coat over the trunk was clipped to a length of about 1/2" and the remainder combed.

During the last part of June and the first part of July the bear's appetite diminished considerably in association with the warm weather but no symptoms of illness were seen other than mild diarrhea. Only hydroponic barley and a little fat was taken. Intake increased again in August as the weather cooled.

On 10/7/80 the bear was again anaethetised and the umbilical area shaved and prepared for surgery. An implanted transmitter was located lying retroperitoneally 3 inches laterally from the implantation site. The incision was sutured and healing was uneventful.

The bear's coat grew rapidly during the recovery period and presently appears in excellent condition. Several scars are visible including ones on the hip and nose. She now consumes approximately 3 lbs. dog chow, 4 lbs. fat and 1 lb. carrots daily.

Summary

When the bear arrived at the Assiniboine Park Zoo she was in more serious condition than had been thought and was deteriorating rather than recovering. Examination and laboratory data revealed that the bear was suffering from renal failure in common with bears 3 and 4. No evidence of neurological disorder was noticed and no areas of spontaneous alopecia were found in the coat.

Treatment was aimed at:

1) Maintaining urinary flow and reducing anaemia
2) Correcting electrolyte disturbances
3) Correcting dehydration

4) Reducing nausea to encourage appetite
5) Inducing a positive metabolic state by providing calories and reducing catabolism of body tissues
6) Reducing anaemia.

Methods of therapy were restricted, owing to the nature of the patient and the necessity for anaesthesia on each occasion. Following the initial examination and treatment, the bear was allowed to adjust to her new surroundings but continued to deteriorate. Once more intensive treatment was initiated, a good clinical response was obtained in terms of the bear's appetite, appearance and laboratory data.

It is likely that when the bear came to the zoo the original precipitating causes had been eliminated but the secondary effects of renal disease and starvation were exacerbating the bear's condition. Treatment allowed the kidneys to heal without further damage. Recovery was gradual and normalisation of biochemical and hematological parameters was concurrent with the clinical improvement.

The development of necrotising infections in the limbs was probably a result of a reduction in the bear's immunity to environmental organisms that gained access during injections through a soiled coat at Churchill and at the zoo. A heathly bear would probably have resisted such inoculations.

The reversibility of the renal disease depends on the nature, location and severity of the lesions, and the rapidity with which the underlying cause is eliminated. The response of this bear, despite its serious condition, offers an excellent prognosis for a complete recovery.

Graham J. Crawshaw
M.S., M.R.C.V.S.
Zoo Veterinarian

Acknowledgement

I am extremely grateful to Drs. Mould, Johnson and Goodridge of Centennial Animal Hospital for their assistance in the treatment of this bear.

Table A6-1

Therapy results

Date	26/3/80	30/3/80	1/4/80	4/4/80	14/4/80	29/5/80	10/7/80
$RBC \times 10^6$/cmm	4.87	2.06	1.81		3.21	5.71	6.55
PCV %	30	15	14	13	19	42.5	49
Hb g. %	11.4	3.5	3.8	4.2	6.1	14.8	17.6
$WBC \times 10^3$/cmm	12.5	4.5	9.6	8.1	20.4	11.8	7.5
Segs. %	94	50	48	82	88	71	55
Bands %	1			1	4	2	3
Lymphs %	4	50	51	16	9	7	21
Mono %	1		1		2	12	2
Eos %						8	18
BUN. mg. %	149	167.5	127	95	36	13.7	11.5
Creatinine mg. %	11.25	10.4	10.8	6.6	2.81		1.59
Glucose mg. %	162	152	272	128	124	120	128
Total protein	11.0	8.0	7.9	6.2	8.7	9.4	8.45
Total Bilirubin mg %	10	4	5.4	2.7	0.9		0.6
Direct " "	7	1.7	1	2.5			
Indirect " "	3	2.3	4.4	0.2			
SGOT u	20	33	190	139	26	43	24
SGPT u	120	12	17	16	12	24	17
Alk. Phos. u	18	27.5	39	22	8.5	18	23
CPK iu/1	106	108	222	83	22	33	26
Amylase u	49	4.0	41		236	74	7.0
Lipase u	1.6	0.6	0.79		0.64	1.05	1.14
Cholesterol mg. %	600	550	500	500	385	400	310
Uric Acid mg. %	2.53		1.01	0.92	1.04	1.57	1.27
Arginase iu/1	4.5	0		0.25		2.9	0
Calcium mg. %	9.95	8.89	8.74	10.15	8.67	9.49	8.87
Phosphorus mg %	6.08	8.31	4.19	6.22	7.04	5.78	4.87
Magnesium mg. %	3.08	2.88	2.85	2.68	2.21	2.26	2.26
Sodium mEq/1	126	120	119	128	134	137	134
Potassium mEq/1	3.6	3.1	2.8	2.7	4.0	4.8	4.8
Chloride mEq/1	71	90	81	93	94	100	102
Albumin gm. %	6.4	4.85				3.61	3.33
α_1 Globulin gm. %	0.8	0.56				0.52	0.70
α_2 Globulin gm. %	0.7	0.56				0.92	0.88
β Globulin gm. %	1.2	0.94				1.58	1.35
γ Globulin gm. %	1.9	1.13				2.78	1.61
A/G ratio	1.39	1.54				0.95	0.66

Table A6-2 cont...2

Date	Behavior	Intake	Treatment
6/4/80	Eyes less sunken	1 lb. liver, 1/4 lb. kidney, 1/2 lb. meat 9 smelt, 12 pieces dog chow	
7/4/80	Abscess draining right foreleg	1/2 lb. meat, 1 1/2 lb. 14 smelt, 12 pieces dog chow 1 carrot	Amoxicillin 1500 mg.
8/4/80		15 smelt, 1 lb. meat, 1 lb. liver, 1/4 lb. dog chow	Amoxicillin 1500 mg.
9/4/80	Slow, dyspneic weak dragging hind feet	14 smelt, 1 lb. meat 1/4 lb. dog chow	Amoxicillin 1500 mg.
10/4/80	Quite bright. Respiration improved	5 lbs. fat, 1 1/2 lb. carrots	Amoxicillin 3000 mg.
11/4/80	Quite aggressive	2 lbs. fat, 10 smelt, 1 can of sardines, 2 carrots, dog chow	Amoxicillin 3000 mg.
12/4/80	Very weak on hind legs	8 smelt, 2 lbs. fat, 1 carrot, dog chow	Trimethoprim/Sulphadiazine KCl 50 mEq
13/4/80	Lame left foreleg Difficulty in moving around	1/4 lb. fat, 4 smelt 1 carrot	Trimethoprim/Sulphadiazine
14/4/80	Not using left leg	10 smelt, 2 1/2 lb. fat 10 pieces dog chow	Anesthesia (1200 mg. ketamine and 400 mg. xylazine), Abscesses cleaned. 2 l. saline s/c, 5 ml. vitamin A, D, E, Trimethoprim
15/4/80	Still not using left leg. Eating less	1 carrot, 2 lbs. fat, dog chow	Trimethoprim/Sulphadiazine

TABLE A6-2

Daily log Bear # 2

Date	Behavior	Intake	Treatment
26/3/80	Depressed, inactive	Water, milk, Protogest	Anesthesia (1300 Mg. ketamine and 300 mg. xylazine), 2 l. lactated Ringer's/5 % dextrose i/v, 2 l. lactated Ringer's s/c, 12 ml. benzathine penicillin, 5 ml. multi vitamins, 1 ml. Equipoise. Neck transmitter removed.
27/3/80	Livelier, alert and aggressive	1 piece of fish	Na Cl in water
28/3/80			'Diaglo' in water
29/3/80	Highly depressed Lying down	Glucose by stomach tube	Anesthesia (1200 mg. ketamine and 300 mg xylazine), 2 l. saline, 250 ml. 50 % glucose i/v, 2 l. saline s/c, 12 ml. benzathine penicillin, 5 ml. multivitamin (additional 5600 mg. ketamine required), Maalox orally.
30/3/80	No change		
1/4/80	More active, growling	Glucose, creamed wheat by stomach tube.	Anesthesia (1200 mg. ketamine and 400 mg. ylazine), 4.1 saline s/c, 1 l. saline i/v, 5 ml. multivitamin, 12 ml. benzathine penicillin.
2/4/80	a.m. livelier, pacing around cage, p.m. lethargic, sitting with head down	2 1/2 smelt	
3/4/80		2 smelt	
4/4/80	Improved	Glucose, creamed wheat by stomach tube	Anesthesia (1200 mg. ketamine and 400 mg. xylazine), 2.5 l. saline s/c kaopectate orally; bear washed.
5/4/80		7 smelts, 1/4 lb heart, 6 pieces dog chow	

Table A6-2 cont...2

Date	Behavior	Intake	Treatment
16/4/80	Quite lively. Pus draining left and right sides		Trimethoprim/Sylphadiazine
17/4/80	Alert and aggressive, discharges reduced		a.m. Trimethopim/Sulphadiazine p.m. Carbenicillin 5g.
18/4/80	Aggressive and eating well	3 lbs. fat, dog chow	Carbenicillin 10g.
19/4/80		3 lbs. fat, dog chow	Carbenicillin 10g.
20/4/80	Right leg draining	"	"
21/4/80	Lame right foreleg draining	"	"
22/4/80	Discharges reduced from right hind leg, right foreleg and neck	"	"
22/5/80	Abscess on left hind leg.		
23/5/80	Abscess on nose		
29/5/80	Transferred		Anesthesia 500 mg. xylazine, 1000 mg. ketamine, 12 ml. benzathine penicillin. Wounds cleaned, coat clipped and combed.
10/7/80			Anesthesia 1200 mg. ketamine and 400 mg. xylazine. Abdominal transmitter removed.